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# A HANDBOOK OF STATISTICS

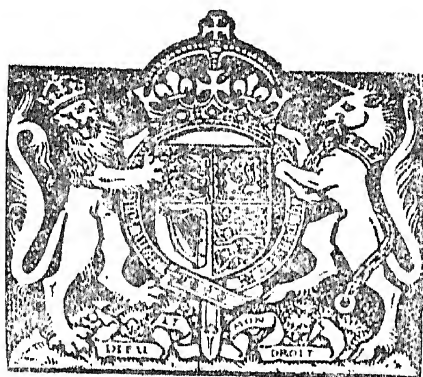
FOR USE IN

## PLANT BREEDING AND AGRICULTURAL PROBLEMS

BY

**F. J. F. SHAW, C.I.E., D.Sc., A.R.C.S., F.L.S.,**

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## FOREWORD

The application of statistical tests to experimental results is a subject the importance of which for the biologist has greatly increased in recent years. Modern statistical text books, however, are generally written by mathematicians and describe the subject in a manner which is obscure to the biological student whose knowledge of mathematics is elementary. It is hoped that this book will provide an easy guide for the students in Indian universities and agricultural colleges and for workers in agricultural departments, in the application of statistical methods to the class of experiments which commonly confront the worker in plant-breeding and agricultural problems. It is not intended as a treatise in mathematical statistics but aims at giving in simple language the methods by which statistics may be used to test the significance of the results of experiments. The book is based on a part of the post-graduate course in plant-breeding which is given in the Botanical Section of the Imperial Institute of Agricultural Research, Pusa, and all the examples given in the book are taken from experiments actually carried out in India and Burma.

The mathematical tables which are necessary for the use of the student who is studying statistics are being published by the Imperial Council of Agricultural Research, and will be readily available for workers in India.

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### CHAPTER I

#### SAMPLING

Statistics is a branch of applied mathematics which deals with observations. In biology our observations deal with living things, either the size of parts or organisms or their frequency of occurrence, and the word BIOMETRY is applied to this branch of statistics. Galton, Pearson, Udney Yule, Fisher, Pearl and Elderton are among the foremost names in the development of what is a relatively young branch of biological science.

In any series of observations it is obviously impossible that our observations should include all the individuals in the universe or even all the individuals in that population which is accessible. Observations are, therefore, based on samples taken from a population and our first care must be that the sample is a true representative of the population.

Samples consist of a number of variables which may be quantitative or qualitative, continuous or discrete. Any quantity or quality which changes is called the variable. The observations which represent the changes in the variable are called variates. A quantitative variate can be expressed by a number, *e.g.*, height of men in inches; in a sample of the males of a population a man 68 inches tall is a variate of 68 inches. A qualitative variate is distinguished by some quality; for example, in a crop of hybrid linseeds individuals with coloured petals can be differentiated from individuals with white petals, and the frequency of occurrence of each class estimated. Quantitative variates are generally *continuous*, that is to say, they exhibit all gradations in size development throughout the sample. *Discrete* variates differ from one another by finite gradations without any intermediate stages, so that each variate has a distinct and separate value and fractions of a unit cannot occur,

*e.g.*, number of petals in a flower, number of Europeans in India, etc. From a sample we can calculate a number of statistical constants (*e.g.*, the average) which convey an idea of the nature of the sample and which summarize briefly its characters.

### THEORY OF SAMPLING

A sample is taken to yield information about a larger bulk or population of individuals, and in order that it may be truly representative of the population it must be so chosen that each individual in the population has an equal and independent chance of being included. A statistical enquiry into the height of men in India could not be carried out on a sample taken exclusively in Bihar, since owing to racial and geographical factors a sample taken from Bihar is not truly representative of the whole population of India. Again a sample of a thousand men which consisted of 500 pairs of brothers would not satisfy the requirements of a true sample, since there is a tendency for brothers to be alike, in other words, in such a sample the variates would be dependent on one another. The conditions under which a sample may be expected to present the characters of the universe from which it has been chosen are :—

(i) *Independence*. The variates which make up the population must be independent of one another and must each have the same chance of inclusion in the sample.

(ii) *Homogeneity*. The universe from which the sample is taken should consist of individuals of the same kind under similar conditions.

### METHODS OF SAMPLING

(1) *Random selection*. This means selection in such a way that every individual in the universe to be sampled has an equal chance of inclusion in the sample. This could be achieved by allocating numbers to all individuals in the universe and drawing numbered tickets blindly from a bag, the numbers so drawn indicating the individuals to be included in the sample. It is not, however, always practicable to number all the individuals in a universe, for instance, if it is desired to sample the heads in a field of wheat the labour involved in allocating a number to every head in a field of only one acre is obviously prohibitive. Under these circumstances the method pursued at Pusa is to make a random selection of heads from the field which is numerically much greater than the sample that is desired. These heads are then numbered and a random drawing of numbers is taken. If a sample of 400 ear-heads is desired the practice at Pusa is to make a random collection of 1,200 heads from the field. It is obvious that in such cases much depends on the true randomness of the original collection in the field. Our method is to set men to walk diagonally across and up and down the field, each man plucking a head on his right or left hand at every pace without any conscious selection. Men are trained to stoop and pluck a stalk from near the ground in order that individuals of short stature may have a fair chance of inclusion in the collection.

A word of caution is necessary with regard to the drawing of the numbered tickets. When the bag contains 1,200 tickets, the odds are 1,199 : 1 against the

drawing of any particular variate, but when 390 tickets have been withdrawn the odds are only 809 : 1 against any particular number being taken. It is, therefore, sometimes desirable to cancel a ticket when drawn and to replace it in the bag so as to keep the total number of tickets in the bag constant. If a cancelled ticket is drawn a second time, the drawing is neglected.

(2) *Spatial selection.* The selection of individuals at regular intervals. This method is suitable to sampling when the universe to be sampled consists of a field in which plants are sown in regular lines. It is then possible to select every 5th, 10th, or 20th plant in the lines according to the size of sample desired. If more convenient, selection by measurement—taking 5, 10 or 20 feet as the space between selected individuals—can also be done.

(3) *Selection by design.* If the universe to be sampled is not homogeneous then the sample should contain members from each of the classes constituting the universe in the proportions in which those classes exist in the universe. If a sample is to be taken from the human population in India, individuals from every race must be included in the sample and the proportion in the sample of any one race should be the same as the proportion of that race in the entire population.

#### RELIABILITY OF THE SAMPLE

If two random samples from the same population yield on analysis statistical constants such as arithmetical averages, which differ significantly, then we infer that either—

- (a) there have been errors in the method of sampling, or
- (b) the samples have been very heterogeneous, or
- (c) the samples are not sufficiently large.

As will be seen later in our studies of probabilities, the significance or reliability of a mean or arithmetical average obtained from a sample, as measured by its probable error or by its standard error, varies inversely as the square root of the number of variates in the sample. Thus, to double the precision of an arithmetical average obtained from a sample of 25, we should require to take a sample of a 100, since  $\sqrt{25}=5$  and  $\sqrt{100}=10$ . Similarly, to treble the precision we have to take a sample of 225 variates, since  $\sqrt{225}=15$ .

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Lovitt, W. V., and Holtzclaw, H. F. (1931). Statistics, Chapter III, Prentice-Hall, Inc.  
Tippett, L. H. C. (1931). The Methods of Statistics, Chapter III, Williams and Norgate, Ltd., London.



## CHAPTER II

## FREQUENCY DISTRIBUTIONS AND AVERAGES

When a sample is taken from a population, it is generally taken with the object of studying some particular attribute of the universe to which it belongs. Thus, for instance, if it is desired to study the occurrence of flower-colour in a hybrid population of linseeds, the attribute of each variate to be measured would be the kind of colour present. The observer would be confronted at the conclusion of his observations with a long list of variates and their attributes and his first task would be to classify these variates according to their attributes. The following table gives a classification of a  $F_2$  population of linseed :—

TABLE I

*Distribution of petal colour in a  $F_2$  population of linseeds*

Variable or Class	Number of variates or Class frequency
Blue . .	169
Lilac . .	61
White . .	62
Pink . .	22
TOTAL .	314

In this case the population of 314 has been divided into four classes and the frequency of each class shows the distribution of the character or attribute under study in the population. The variables in this example are discrete, that is to say, the numbers cannot be continuous since fractional parts of a variate cannot occur. The division of the population into classes is easy since each variable is clear and distinct from any of the others. The class frequency of any particular variate is the number of times that variate occurs in the population. / class

The frequency distribution, when the attributes which are to be studied are quantitative, necessitates the classification of a number of numerical quantities which are continuous, *i.e.*, show all gradations of value within a definite range. If, for instance, it is desired to measure the length of head of a variety of wheat, the observer will obtain a large number of measurements from a sample taken with the precautions previously described. His first task will be to classify these data with the object of reducing them to a form in which they can be mathematically handled. The classification of such a mass of data involves the division of the variates into groups, each group or class containing variates of similar values. Table II gives the data of the length of ear-head and the number of grains per ear-head in a sample of Pusa 12 wheat and the frequency distribution of the length of ear-head is shown in Table III, where the 400 variates are grouped into 17 classes.

TABLE II

*Data of length of ear-head and number of grains per ear in Pusa 12 wheat. Sample taken from Jhilli field (Pusa Farm) 1930-31*

Serial number	Length of ear-head	Number of grains per ear-head	Serial number	Length of ear-head	Number of grains per ear-head	Serial number	Length of ear-head	Number of grains per ear-head	Serial number	Length of ear-head	Number of grains per ear-head
1	10.0	25	51	10.2	38	101	10.4	31	151	10.9	32
2	10.7	36	52	8.9	20	102	9.7	40	152	7.8	18
3	9.8	27	53	9.8	26	103	9.7	28	153	10.0	29
4	10.6	32	54	8.4	24	104	10.4	27	154	11.9	37
5	11.2	32	55	8.8	23	105	11.0	40	155	8.7	27
6	9.2	27	56	9.0	28	106	11.2	31	156	9.4	28
7	9.2	31	57	9.5	29	107	11.5	33	157	11.7	32
8	11.2	36	58	13.4	48	108	10.9	31	158	13.1	45
9	8.5	26	59	10.5	33	109	13.6	41	159	9.6	33
10	9.7	32	60	7.7	17	110	11.0	40	160	10.2	40
11	12.5	42	61	10.1	30	111	9.5	23	161	10.4	37
12	8.3	22	62	8.2	29	112	11.1	35	162	9.5	23
13	9.8	32	63	11.4	38	113	10.5	33	163	12.0	40
14	10.4	28	64	7.5	20	114	9.8	31	164	10.6	30
15	10.8	33	65	9.6	34	115	12.2	36	165	8.7	25
16	10.0	30	66	6.6	17	116	8.5	28	166	10.9	31
17	9.6	26	67	9.9	29	117	8.4	33	167	12.2	39
18	8.8	27	68	12.7	42	118	9.3	29	168	8.0	14
19	11.8	42	69	10.2	38	119	9.1	28	169	12.8	50
20	9.5	26	70	9.6	27	120	10.6	29	170	9.7	25
21	9.5	26	71	10.0	28	121	11.5	30	171	6.5	17
22	9.4	27	72	11.7	38	122	10.5	33	172	11.5	35
23	10.0	28	73	8.7	34	123	10.4	24	173	9.8	31
24	10.3	28	74	8.4	30	124	10.4	34	174	10.0	27
25	7.5	14	75	10.0	32	125	9.4	23	175	9.8	32
26	8.4	26	76	8.3	21	126	9.0	22	176	9.4	21
27	11.6	39	77	7.9	20	127	9.4	22	177	6.3	17
28	11.1	34	78	10.0	32	128	11.3	35	178	10.9	29
29	9.5	26	79	10.9	26	129	11.8	34	179	7.6	21
30	11.3	44	80	9.7	32	130	9.6	29	180	7.6	23
31	8.9	24	81	12.5	34	131	8.4	23	181	10.1	33
32	9.4	27	82	10.5	40	132	8.7	26	182	8.9	24
33	10.2	35	83	11.3	40	133	8.5	25	183	10.9	35
34	11.4	32	84	10.8	28	134	11.4	40	184	8.9	31
35	9.9	36	85	10.7	30	135	10.8	34	185	9.4	31
36	10.2	30	86	10.5	32	136	10.6	30	186	5.5	13
37	10.1	28	87	9.5	31	137	10.5	35	187	6.3	16
38	10.4	32	88	9.1	37	138	9.9	23	188	10.1	29
39	9.9	36	89	9.1	27	139	6.7	17	189	8.6	33
40	9.4	28	90	12.1	42	140	9.5	26	190	10.6	38
41	8.9	26	91	12.5	43	141	7.0	17	191	8.8	26
42	12.0	38	92	9.0	32	142	10.6	28	192	9.3	29
43	13.0	41	93	11.2	39	143	11.2	39	193	13.3	38
44	9.7	28	94	9.7	30	144	11.5	45	194	11.5	37
45	8.9	25	95	11.0	32	145	8.3	21	195	11.3	37
46	7.9	19	96	11.1	32	146	8.4	25	196	10.0	30
47	8.9	24	97	9.7	26	147	7.0	15	197	10.7	30
48	9.5	30	98	8.9	25	148	9.6	25	198	9.8	26
49	11.0	35	99	10.1	29	149	11.7	33	199	6.7	15
50	9.8	27	100	11.4	42	150	8.9	28	200	8.5	24

*Data of length of ear-head and number of grains per ear in Pusa 12 wheat. Sample taken from Jhilli field (Pusa Farm) 1930-31—contd.*

Serial number	Length of ear-head	Number of grains per ear-head	Serial number	Length of ear-head	Number of grains per ear-head	Serial number	Length of ear-head	Number of grains per ear-head	Serial number	Length of ear-head	Number of grains per ear-head
201	9.7	31	251	10.0	31	301	8.7	23	351	10.2	31
202	11.7	44	252	8.4	23	302	10.2	30	352	12.1	41
203	8.9	23	253	10.6	29	303	12.0	41	353	6.2	16
204	11.6	30	254	10.6	39	304	10.4	35	354	10.8	35
205	11.7	36	255	9.2	34	305	12.0	42	355	10.9	32
206	9.2	31	256	10.5	34	306	11.9	33	356	11.0	34
207	10.2	31	257	9.4	26	307	8.5	23	357	10.5	34
208	10.6	32	258	10.0	27	308	6.4	14	358	10.0	29
209	10.4	29	259	9.3	32	309	11.4	41	359	11.5	35
210	11.8	41	260	11.2	38	310	9.8	28	360	9.2	28
211	10.6	39	261	8.7	27	311	10.2	28	361	8.0	24
212	10.3	30	262	9.6	34	312	10.9	38	362	11.8	38
213	10.7	26	263	9.2	33	313	10.1	33	363	9.7	28
214	11.8	32	264	9.3	27	314	12.1	41	364	9.0	27
215	11.8	33	265	9.5	34	315	9.5	34	365	6.3	18
216	9.8	30	266	9.8	29	316	10.4	36	366	10.3	41
217	9.9	35	267	10.0	27	317	9.9	39	367	8.6	27
218	9.6	25	268	11.2	31	318	9.2	27	368	10.2	32
219	9.6	31	269	11.0	34	319	11.3	38	369	8.0	23
220	9.6	24	270	11.6	38	320	8.8	26	370	10.0	31
221	13.7	51	271	10.0	37	321	8.4	36	371	10.4	33
222	9.9	26	272	13.2	39	322	8.7	22	372	11.4	33
223	5.7	15	273	7.6	18	323	9.9	26	373	8.9	29
224	9.2	18	274	10.1	34	324	12.1	42	374	11.5	38
225	9.4	32	275	11.4	36	325	10.5	33	375	10.8	36
226	10.7	45	276	12.0	35	326	7.0	21	376	10.5	35
227	8.8	27	277	10.0	40	327	11.5	35	377	11.8	35
228	8.8	25	278	10.3	34	328	9.7	28	378	10.4	31
229	11.1	32	279	10.5	40	329	8.2	21	379	10.4	39
230	10.4	27	280	10.9	43	330	9.6	29	380	9.5	27
231	11.5	32	281	10.8	34	331	11.6	35	381	11.8	42
232	11.9	44	282	9.0	31	332	8.4	23	382	9.8	30
233	10.5	40	283	10.4	28	333	9.5	27	383	8.5	23
234	7.1	19	284	12.3	43	334	8.6	33	384	10.7	35
235	10.8	30	285	9.7	30	335	12.9	40	385	10.0	30
236	9.5	30	286	9.1	30	336	9.2	26	386	10.7	30
237	8.6	35	287	10.0	33	337	10.2	29	387	9.2	24
238	12.2	49	288	8.8	28	338	10.3	32	388	10.8	32
239	11.6	33	289	9.8	28	339	12.4	33	389	10.3	36
240	10.7	42	290	9.7	34	340	9.4	30	390	8.2	22
241	9.0	29	291	5.4	10	341	8.9	25	391	9.5	34
242	8.6	26	292	10.1	29	342	9.9	24	392	6.8	19
243	10.1	32	293	8.3	28	343	9.8	26	393	10.6	36
244	11.2	23	294	9.0	25	344	6.9	21	394	10.0	31
245	9.3	25	295	10.7	38	345	11.8	36	395	12.4	39
246	11.2	38	296	10.0	31	346	13.1	32	396	8.1	24
247	7.9	19	297	9.3	30	347	9.4	25	397	7.5	21
248	9.0	26	298	13.5	54	348	10.5	31	398	10.3	32
249	9.4	29	299	11.0	31	349	13.6	39	399	8.8	27
250	7.4	17	300	10.2	30	350	8.7	21	400	10.2	31

In the measurement of the length of 400 ear-heads, we find that a wide degree of variation exists and that a continuous series of measurements is obtained ranging from one extreme to the other. In this example, the smallest ear-head measures 5.4 cms. and the largest measures 13.7 cms. We, therefore, have a range of 5.3 to 13.7 cms. which can be divided into 17 classes by taking class intervals of 0.5 cm. The mid-point of each class is taken as the class value and the frequency of the class consists of the number of variates which fall within the limits of the class.

TABLE III

*Frequency distribution showing the length of ear-heads in Pusa 12 wheat*

Classes	Class value	Frequency	Frequency $\times$ Class value
1	2	3	4
5.3—5.7	5.5	3	16.5
5.8—6.2	6.0	1	6.0
6.3—6.7	6.5	8	52.0
6.8—7.2	7.0	6	42.0
7.3—7.7	7.5	8	60.0
7.8—8.2	8.0	11	88.0
8.3—8.7	8.5	32	272.0
8.8—9.2	9.0	42	378.0
9.3—9.7	9.5	58	551.0
9.8—10.2	10.0	65	650.0
10.3—10.7	10.5	55	577.5
10.8—11.2	11.0	37	407.0
11.3—11.7	11.5	31	356.5
11.8—12.2	12.0	24	288.0
12.3—12.7	12.5	7	87.5
12.8—13.2	13.0	6	78.0
13.3—13.7	13.5	6	81.0
TOTAL	..	400	3991.0

The extreme classes contain very few variates, *i.e.*, their frequencies are very low, and in the middle classes the variates are more numerous, *i.e.*, the frequencies are high. The student must exercise caution in classifying those variates whose values fall about the limits of each class-range. For instance, the first class includes

variates of 5.3 or above up to and including variates of 5.7, variates of 5.8 fall into the second class and similarly variates of 6.3 fall into the third class.

#### CLASS INTERVAL

In choosing a class interval certain important considerations should be borne in mind.

- (1) The class interval must be of uniform width and of such size that the characteristic features of the distribution are displayed. Thus, the class interval must not be so large that a considerable error would be involved in assuming that the mid-point of the interval is the average of the class. It must not be so small as to give classes with zero frequencies, or frequencies approaching zero.
- (2) The range of the classes should cover the entire range of the group and the classes must be continuous.
- (3) As a general rule, the number of classes should be about 15 and never more than thirty nor less than six.
- (4) It is a convenience to make the mid-point of a class a whole number.

#### GRAPHIC REPRESENTATION OF FREQUENCY DISTRIBUTIONS

The information contained in Table III can also be expressed as a graph and indeed this method permits of a ready grasp of certain important features which are common to some types of frequency distributions. The graph is obtained by plotting class values as abscissæ and class frequencies as ordinates. A curve is then obtained in which the maximum frequencies are at the middle of the range and the class frequencies diminish more or less symmetrically in the direction of the extremes (Fig. 1).

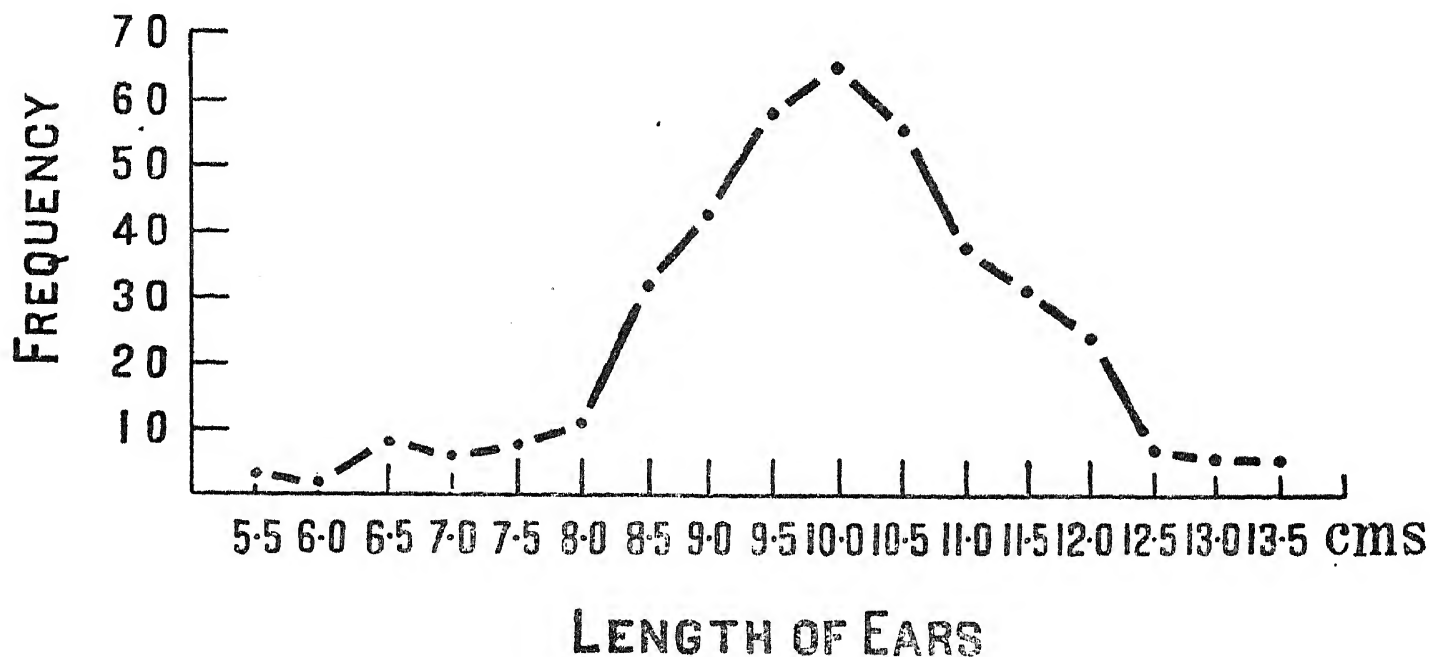


Fig. 1.—Frequency curve of length of ear-heads of Pusa 12 wheat in a sample of 400.

Another graphical method of depicting frequency distributions is to measure along the horizontal axis distances proportional to the class intervals and to raise on each of these distances rectangles proportional in height to the number of individuals falling within the class; the resulting figure is called a histogram. The two methods are practically equivalent when the class intervals are equal, as they always are, in the simple statistical problems with which this book deals.

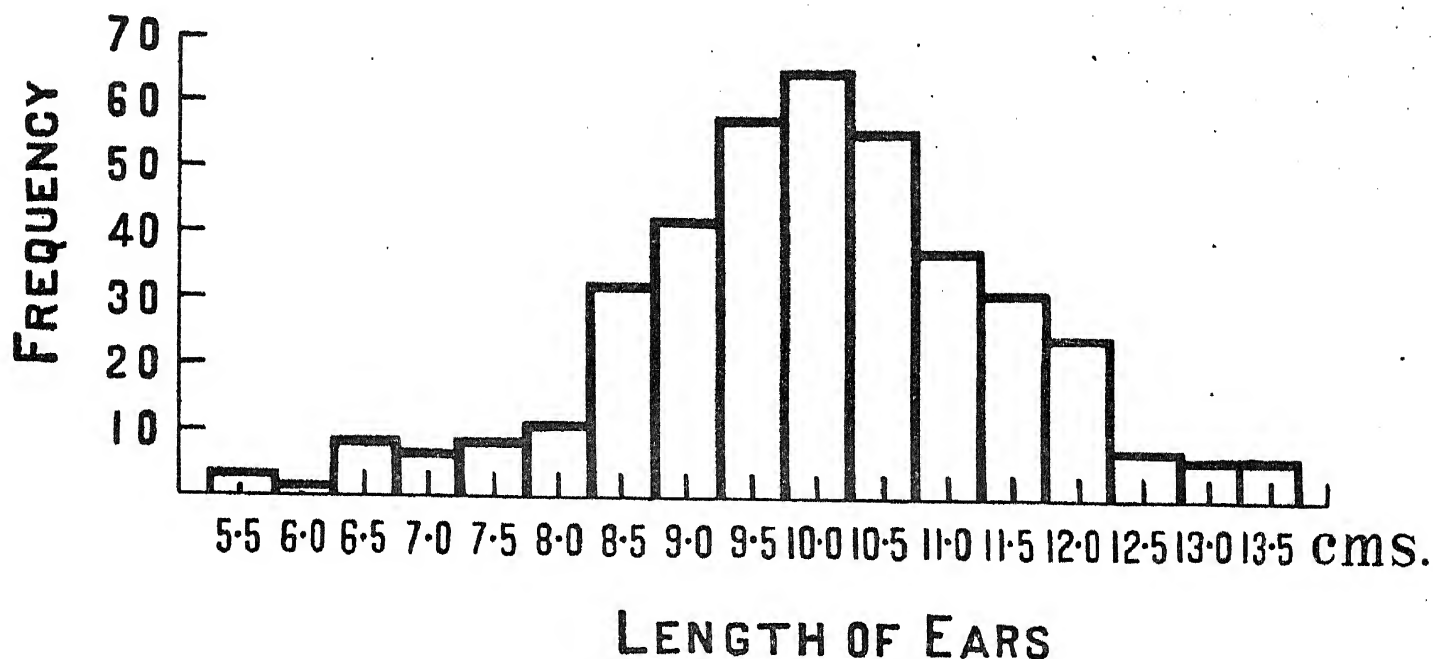


Fig. 2.—Histogram showing the distribution of the length of ear-head of Pusa 12 wheat.

### AVERAGES

From the frequency table certain biometrical constants can be calculated which summarize the main features of the data.

(1) *Mean.* The mean is the arithmetic average and is the result obtained when the sum of the measurements of the items in a sample is divided by the number of items in the sample. When a frequency table is available the mean can be calculated from the formula

$$M = \frac{\sum f.V}{n} \quad \dots \dots \dots (1)$$

where  $f$  = the class frequency,  $V$  = the class value,  $n$  = the size of the sample and  $\Sigma$  indicates the summation of the products of all values of  $f$  and  $V$ .

Substituting in this formula the values in the example in Table III, we obtain

$$M = \frac{3991.0}{400} = 9.9775 \text{ cms.}$$

The mean in this case, therefore, is 9.9775 cms.

The mean is simply an average and tells us nothing of the distribution, relative size or number of the items. Thus each of the following series :—

7 7 7 7 7  
 5 6 7 8 9  
 4 5 6 8 9 10  
 1 1 2 2 3 5 35  
 2 12

has 7 as an arithmetic average.

The mean is useful because it gives weight to all items in direct proportion to their size and lends itself to algebraic treatment. Thus the averages of two or more series may be obtained from the averages of the individual series and the algebraic sum of *plus* and *minus* deviations from the mean is zero.

(2) *Weighted mean*. It sometimes happens that some determinations of the mean are made under more favourable conditions than others and are, therefore, esteemed as of greater reliability. More importance is attached to such determinations by considering each observation to be equal to at least 2 or 3 ordinary determinations. That is to say, that the weight of such a determination of the mean is equal to 2 or 3 determinations.

(3) *Mode*. The mode is the size of that variate which occurs most frequently. In a frequency table the modal class is the class which has the greatest frequency. This class can be determined at once from inspection, but the true value of the mode will be located somewhere in that class interval, not necessarily at the mid-point of the class.

(4) *Median*. The median is the value which is located in the middle of a series when the items are arranged in order of magnitude and which divides the series into two equal parts so far as the number of items in the series is concerned. The determination of the median is a simple matter when there is an odd number of items in the series. Thus, if 101 items are placed in the order of their magnitude, the 51st item will be the value of the median. If there are an even number of items in a series, the average of the two central values may be taken as expressing the median. In a frequency distribution such as we have described for wheat in which the curve expressing the distribution approaches a symmetrical form, the median will be the value of that ordinate which divides the curve into two equal areas.

(5) *Range*. The total range of a distribution is given by the difference between the smallest and the largest variates and is an indication of the dispersion or variability of the distribution. In our wheat example the range is from 5.3 cms. to 13.7 cms. and the variation in the length of ear-heads is, therefore, between these two limits.

#### FREQUENCY CURVE

The frequency graph for a series distributed almost symmetrically about the mean approaches more and more to a smooth curve as the number of items in the

series increases, *i.e.*, as the sample gets larger. It can be shown mathematically that with an infinite number of items a perfectly symmetrical curve is produced which is representative of the successive terms of the expansion of the binomial  $(a + b)^n$  when  $a = b = 1$  and  $n$  is any integer. Thus—

$$(a + b)^1 = 1 + 1$$

$$(a + b)^2 = 1 + 2 + 1$$

$$(a + b)^4 = 1 + 4 + 6 + 4 + 1$$

$$(a + b)^6 = 1 + 6 + 15 + 20 + 15 + 6 + 1$$

$$(a + b)^{10} = 1 + 10 + 45 + 120 + 210 + 252 + 210 + 120 + 45 + 10 + 1.$$

Such a perfectly symmetrical curve is called the *normal curve*, and in it the mean, the median and the mode coincide and divide the curve into two equal areas, half the total number of items being in each half of the curve.

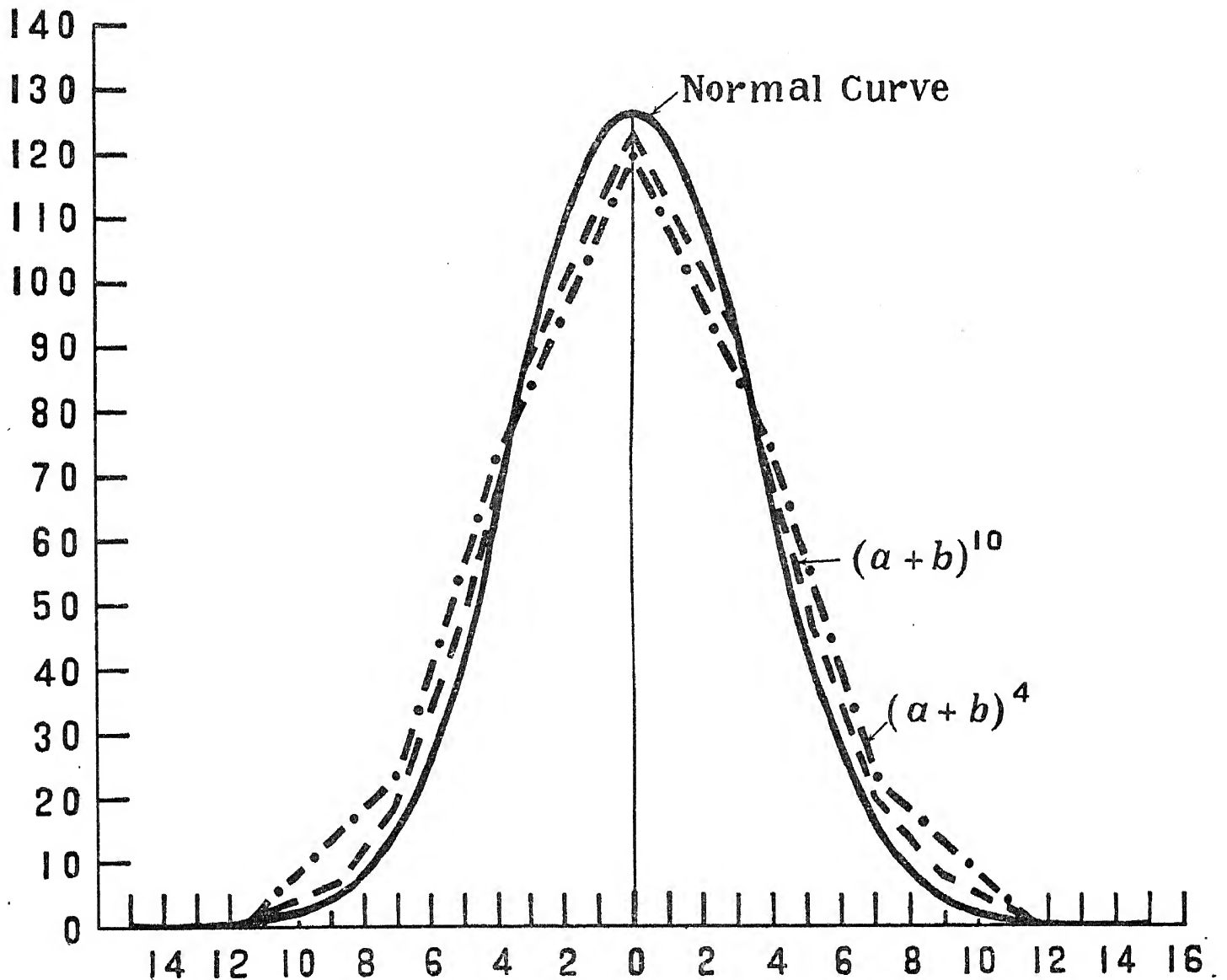


Fig. 3.—The normal curve and curves for values of  $(a + b)^n$ .



The slope of the curve is an indication of the amount of variability in the sample and the width at the point of greatest breadth indicates the range of variability. The steeper the slope of the curve, the less the amount of variation in the sample. The ordinates which divide each half of the total area of the curve into two equal parts are called the *quartiles* and with the median divide the curve into four equal areas, that is to say, in a perfectly symmetrical distribution the median, with which the mean coincides, and the quartiles divide the items into four numerically equal groups. The steeper the slope of the curve, the shorter is the distance between a quartile and the mean, and hence the distance between the quartile and the mean may be used as a measure of variability.

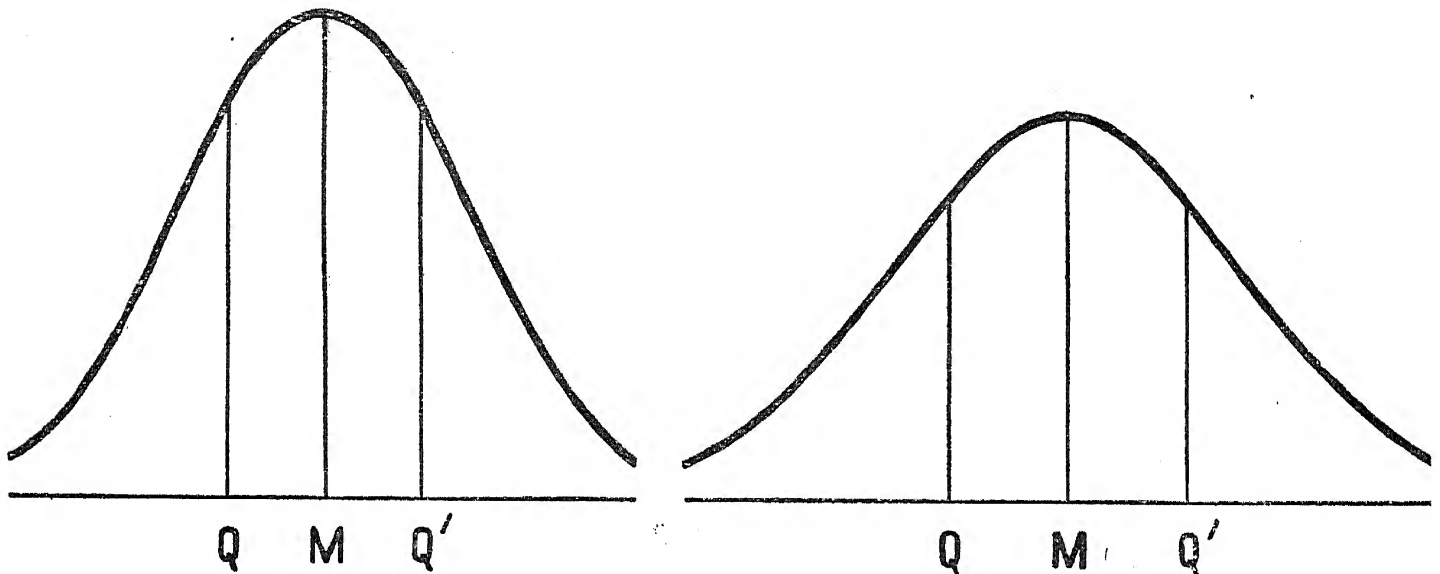


Fig. 4.—Normal curves showing means and quartiles.

Fig. 4 above illustrates the relationship of the mean and the quartiles and the slope of the curves in two curves of similar area. Curve on the right hand represents a sample with a larger amount of variability than the curve on the left. In each curve the distance

$$MQ = MQ'.$$

Curves based on adequate samples from a homogeneous population are generally unimodal. If the curve shows more than one mode, this is an indication of lack of uniformity in the sample. In the case of the measurements of a biological variable such as length of ear-head in wheat, the presence of a multimodal curve would lead us to infer that the sample had been taken from a mixture of different types or that the sample was inadequate in size. Of course, no curve based on measurements of a biological variable will exhibit the perfect symmetry of the normal curve but if the sampling has been adequate and the population is uniform, the departure from symmetry is not such as to preclude the application of the statistical principles of the normal curve to the solution of such problems. When curves are not symmetrical, the mean and the mode will not coincide, and such curves are said to show skewness.

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## CHAPTER III

## MEASUREMENT OF DISPERSION

The mean of a sample is a measure of the type which constitutes the population, it tells us, however, nothing as to the extent and nature of the variability or dispersion within the type, nor do the mode and the median by themselves give us any estimation of dispersion. In any sample which follows a normal distribution, the variates are dispersed about the mean in a more or less symmetrical manner. The limits of dispersion are marked by the smallest and the largest variates and give us the range of the variability. As already explained, the slope of the frequency curve furnishes an indication of the degree and the nature of dispersion but since curves based on actual samples are never absolutely symmetrical, the mean and the median in such curves do not coincide and the quartiles in such curves are not equidistant from the median and therefore do not afford a satisfactory index of variability; moreover, they are reckoned with reference to the median and not to the mean and it is usual and preferable in actual samples to calculate the dispersion about the mean. For these reasons, the measure of variability in use is not the quartile but is the *standard deviation*, a function which treats deviations above and below the mean on the same terms. The standard deviation is a measure of dispersion from the arithmetic mean and is calculated by squaring the deviation of each item from the mean, summing the squares, dividing by the number of observations and then extracting the square root, as shown in the following formula:—

$$\sigma = \sqrt{\frac{\sum f.d^2}{n}} \quad . \quad . \quad (2)$$

where  $\sigma$  = the standard deviation,  
 $f$  = class frequency,  
 $d$  = the deviation of the class value from the mean,  
 $\Sigma$  = the symbol for summation of all values of  $f \times d^2$ ,  
 and  $n$  = the number of variates in the sample.

Since both negative deviations below the mean and the positive deviations above the mean are squared, the products of  $f$  and  $d^2$  are always positive numbers.

The student must remember that the standard deviation is an absolute measure of dispersion and is expressed in terms of the unit of measurements, *e.g.*, grammes, inches, pounds, etc.

The term variance is used to denote the square of the standard deviation, *i.e.*,

$$\sigma^2 = \frac{\sum f.d^2}{n}$$

A simpler measure of variability is afforded by the *average or mean deviation*. This is calculated by summing the deviations from the mean irrespective of the sign and dividing by the number of observations. The average deviation is inferior to the standard deviation because the squaring of deviations in the latter case gives

more adequate representation to the extreme variates which are generally of low frequencies. The formula for calculating the average deviation is—

$$A. D. = \frac{\sum f|d|}{n} \quad . \quad . \quad (3)$$

The comparison of two standard deviations of different samples, however, affords little indication of the relative amounts of variation in the two samples. For instance, a standard deviation of 2 in a sample with a mean of 100 actually indicates a smaller amount of variation than a standard deviation of 0.5 in a sample with a mean of 10. It is obvious that the quantity 2 considered in relation to the mean of 100 is smaller than the quantity 0.5 in relation to the mean of 10. In comparing, therefore, the amounts of variation in two distributions, it is desirable to take account of the relative size of the items. It must also be remembered that the variation as measured by the standard deviation may be expressed in different units, e.g., grammes, inches, pounds, etc., in different distributions and, therefore, it is desirable to have a relative measure of variation when comparing several samples. It is usual to make such a comparison by means of the *coefficient of variation* which is a percentage ratio of the standard deviation to the mean, according to the following formula :—

$$C. V. = \frac{\sigma}{M} \times 100 \quad . \quad . \quad (4)$$

Applying this formula to our example of the length of ear-head in Pusa 12 wheat, we have—

$$M = 9.9775 \text{ cms.}$$

$$\sigma = 1.4408 \text{ cms.}$$

$$\text{therefore } C. V. = \frac{1.4408}{9.9775} \times 100 = 14.44 \text{ per cent.}$$

and we may say that the coefficient of variation is about 14 per cent.

The use of the coefficient of variation, as a more reliable indication of the amount of variability than the standard deviation, is shown in the following example of the average yields of B. S. 1 oats in two successive years at Karnal in plots of equal area with five replications.

Year		Mean lb.	Std. dev. lb.	Coeff. var. %
1931-32	. . . . .	31.80	7.527	23.67
1932-33	. . . . .	55.90	7.838	14.02

The values of the two standard deviations do not differ widely but considered in relation to their respective means it is evident that the amount of variability in the second year as measured by the coefficient of variation is much smaller than in the first year.

#### METHODS OF CALCULATING MEAN, STANDARD DEVIATION AND COEFFICIENT OF VARIATION

(1) *The ordinary method.*—This method is sometimes called the long method and is simply the straightforward application of the principles described in the

preceding pages. Table IV gives the details of the calculations for the number of grains per ear in Pusa 12 wheat. The data from Table II are first arranged in a frequency table as in the previous example of the length of ear-heads; in the case of the number of grains per ear we are dealing with a discrete variable since fractions of a grain cannot occur, the class limits will, therefore, run 8-12, 13-17, 18-22, ..... and there is little possibility of variates which occur near the class limits, being wrongly classified. In Table IV, columns 1, 2, 3 and 4 give the data necessary for the calculation of the mean and are the same as Table III; columns 5-7 deal with the deviation,  $d$ , of each class value from the mean and the summation of the products of  $f.d^2$ , the standard deviation is calculated from the formula already given but a correction factor has to be applied to compensate for the error introduced by grouping variates into classes and basing the calculations on the values of class centres. The reason for this correction, which is called *Sheppard's Correction*, is that the class centres may not be mid-points of the distributions of the variates within the classes and yet this is the assumption which is made when we group the variates into classes, and base our calculations on the class centres without applying any correction. Sheppard's correction for standard deviations is  $\frac{1}{4}$ th of the <sup>square of the</sup> class interval and is to be deducted from the term  $\frac{\sum f.d^2}{n}$  as is shown in the following example where Sheppard's correction amounts to 2.0833.

TABLE IV

*Calculation of mean and standard deviation of the Number of grains per ear-head in Pusa 12 wheat*

Class	Class value	Frequency	Frequency × Class value	Deviation from the mean	Deviation squared	Frequency × Deviation squared
$C$	$V$	$f$	$f.v$	$d$	$d^2$	$f.d^2$
1	2	3	4	5	6	7
8—12 . .	10	1	10	—20.55	422.3025	422.3025
13—17 . .	15	17	255	—15.55	241.8025	4110.6425
18—22 . .	20	25	500	—10.55	111.3025	2782.5625
23—27 . .	25	86	2150	—5.55	30.8025	2649.0150
28—32 . .	30	125	3750	—0.55	0.3025	37.8125
33—37 . .	35	77	2695	+4.45	19.8025	1524.7925
38—42 . .	40	55	2200	+9.45	89.3025	4911.6375
43—47 . .	45	9	405	+14.45	208.8025	1879.2225
48—52 . .	50	4	200	+19.45	378.3025	1513.2100
53—57 . .	55	1	55	+24.45	597.8025	597.8025
TOTAL .	..	400	12220	..	..	20429.0000

$$M = \frac{\sum f.v}{n} = \frac{12220}{400} = 30.55$$

$$\sigma = \sqrt{\frac{\sum f.d^2}{n} - \left(\frac{1}{12} \text{ of } i^2\right)} \quad \text{where } i = \text{class interval}$$

$$= \sqrt{\frac{20429}{400} - 2.0833} = 6.9992$$

$$C. V. = \frac{\sigma}{M} \times 100 = \frac{6.9992}{30.55} \times 100 = 22.911 \text{ per cent of the mean.}$$

The frequency curve of the distribution of number of grains per ear-head is shown in Fig. 5.

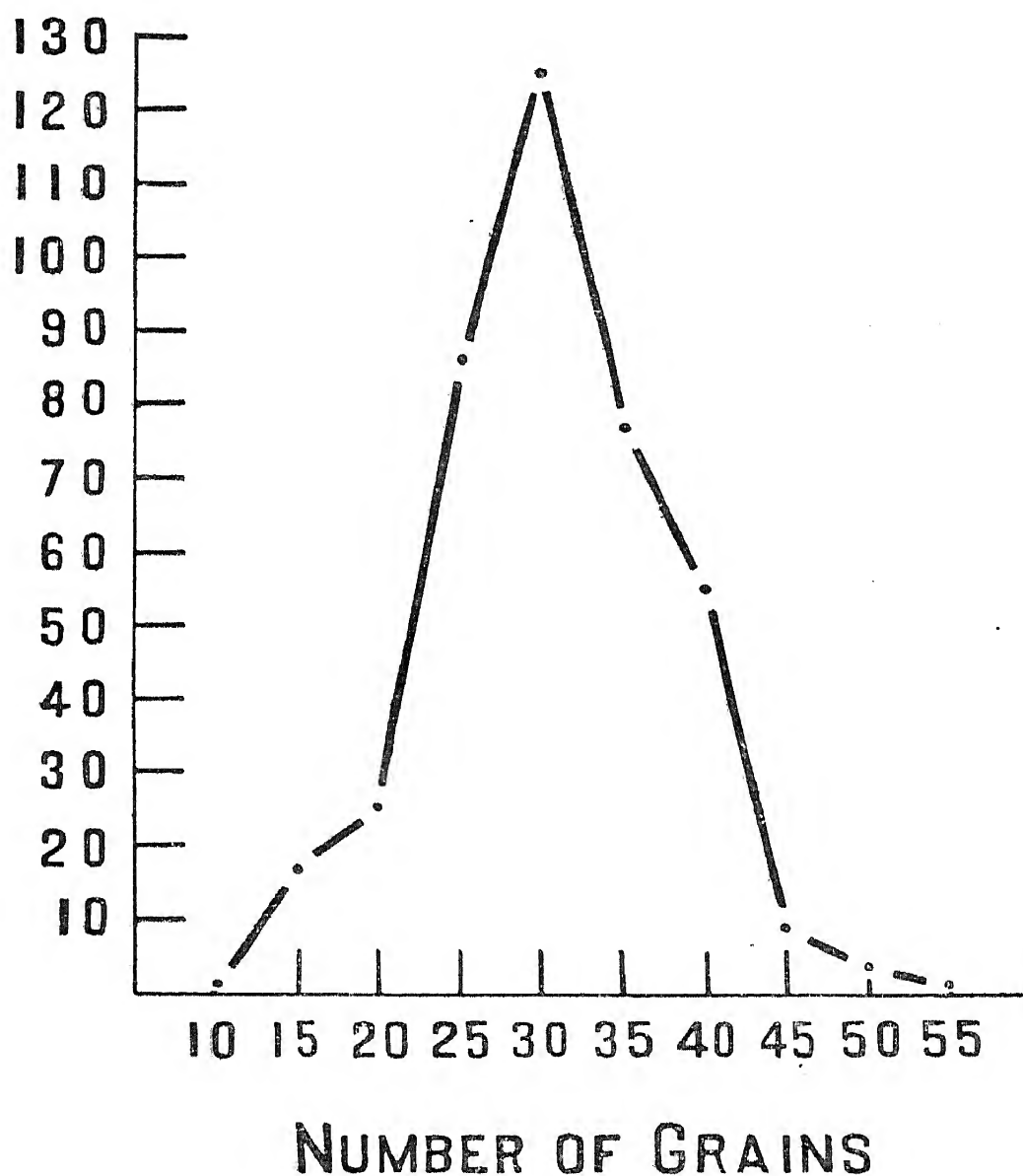


Fig. 5.—Frequency curve of the number of grains per ear-head in Pusa 12 wheat.

(2) *The short method.*—The long and tedious calculation of the preceding example may be considerably shortened by the use of the short method illustrated in Tables V and VI. In this method a convenient assumed value, which must agree with one of the class values, is taken as the mean and is referred to under the letters *A. O.* meaning arbitrary origin. The deviations,  $d'$  from this arbitrary origin are

taken as shown in column 4 in Table V without reference to the class values and are expressed as units of class intervals ; thus the deviation,  $d'$ , of the last class is given as 9 because this class is 9 class-intervals removed from the class which contains the *A. O.* For this last class the frequency is 1 and therefore  $f (d')^2 = 1 \times (9)^2$ . The products  $f.d'$  and  $f.d'^2$  are given in columns 5 and 6. Since the calculations have been based not on the *true mean* but upon the arbitrary origin and since the deviations are expressed as units of class intervals, corrections have to be applied to the arbitrary origin to determine the mean and to the usual formula to determine the standard deviation. Thus—

$$M = A.O. + i \left( \frac{\sum f.d'}{n} \right) \quad . \quad . \quad . \quad . \quad . \quad (5)$$

$$\text{and } \sigma = \sqrt{\left\{ \frac{\sum f.d'^2}{n} - \left( \frac{\sum f.d'}{n} \right)^2 \right\} \times i^2 - \left( \frac{1}{12} \text{ of } i^2 \right)}. \quad . \quad (6)$$

A word of caution seems necessary in applying Sheppard's correction. This correction is  $\frac{1}{12}$ th of the class interval squared and must invariably be deducted from the variance after the necessary corrections have been made as shown in Formula 6.

In this example the class value of the first class has been taken as the arbitrary origin and all deviations,  $d'$ , are positive, this, however, is not necessary and a class value in the middle or in any other part of the distribution may be taken as the arbitrary origin, in which case the lower classes will give negative deviations and the higher classes will give positive deviations. This is done in Table VI which gives the calculations of mean and standard deviation for the length of ear-head in Pusa 12 wheat by the short method.

TABLE V

*Calculation of mean and standard deviation of the number of grains per ear-head in Pusa 12 wheat*

Class	Class value	Frequency	Deviations from arbitrary origin	Frequency $\times$ Deviation	Frequency $\times$ Deviation squared
<i>C</i>	<i>V</i>	<i>f</i>	<i>d'</i>	<i>f.d'</i>	<i>f.d'^2</i>
1	2	3	4	5	6
8—12 . .	10	1	0	0	0
13—17 . .	15	17	1	17	17
18—22 . .	20	25	2	50	100
23—27 . .	25	86	3	258	774
28—32 . .	30	125	4	500	2000
33—37 . .	35	77	5	385	1925
38—42 . .	40	55	6	330	1980
43—47 . .	45	9	7	63	441
48—52 . .	50	4	8	32	256
53—57 . .	55	1	9	9	81
TOTAL .	..	400	..	1644	7574

$$i = 5$$

$$A. O. = 10$$

$$\text{Correction factor} = \frac{\sum f.d'}{n}$$

$$M = A. O. + i \times \frac{\sum f.d'}{n}$$

$$= 10 + 5 \times \frac{1644}{400} = 30.55$$

$$\sigma = \sqrt{\left\{ \frac{\sum f.d'^2}{n} - \left( \frac{\sum f.d'}{n} \right)^2 \right\} \times i^2 - \left( \frac{1}{12} \text{ of } i^2 \right)}$$

$$= \sqrt{\left\{ \frac{7574}{400} - \left( \frac{1644}{400} \right)^2 \right\} \times 25 - \left( \frac{1}{12} \text{ of } 5^2 \right)}$$

$$= 6.9992$$

$$C. V. = \frac{\sigma}{M} \times 100 = \frac{6.9992 \times 100}{30.55} = 22.911 \text{ per cent.}$$

TABLE VI

*Calculation of mean and standard deviation of the length of ear-head in Pusa 12 wheat*

Class	Class value	Frequency	Deviations from arbitrary origin	Frequency × Deviation	Frequency × Deviation squared
<i>C</i>	<i>V</i>	<i>f</i>	<i>d'</i>	<i>f.d'</i>	<i>f.d'^2</i>
1	2	3	4	5	6
5.3—5.7 . .	5.5	3	—8	—24	192
5.8—6.2 . .	6.0	1	—7	—7	49
6.3—6.7 . .	6.5	8	—6	—48	288
6.8—7.2 . .	7.0	6	—5	—30	150
7.3—7.7 . .	7.5	8	—4	—32	128
7.8—8.2 . .	8.0	11	—3	—33	99
8.3—8.7 . .	8.5	32	—2	—64	128
8.8—9.2 . .	9.0	42	—1	—42	42
9.3—9.7 . .	9.5	58	0	0	0
9.8—10.2 . .	10.0	65	+1	+65	65
10.3—10.7 . .	10.5	55	+2	+110	220
10.8—11.2 . .	11.0	37	+3	+111	333
11.3—11.7 . .	11.5	31	+4	+124	496
11.8—12.2 . .	12.0	24	+5	+120	600
12.3—12.7 . .	12.5	7	+6	+42	252
12.8—13.2 . .	13.0	6	+7	+42	294
13.3—13.7 . .	13.5	6	+8	+48	384
TOTAL .	..	400	..	—280 +662 = +382	3720



$$i = 0.5 \text{ cms.}$$

$$A. O. = 9.5 \text{ cms.}$$

$$\text{Correction factor} = \frac{\sum f.d'}{n} \text{ for determining true Mean.}$$

$$,, \quad ,, = \left( \frac{\sum f.d'}{n} \right)^2 \text{ for determining standard deviation.}$$

$$M = A. O. + i \times \frac{\sum f.d'}{n}$$

$$= 9.5 + 0.5 \times \frac{382}{400} = 9.9775$$

$$\sigma = \sqrt{\left\{ \frac{\sum f.d'^2}{n} - \left( \frac{\sum f.d'}{n} \right)^2 \right\} \times i^2 - \left( \frac{1}{12} \text{ of } i^2 \right)}$$

$$= \sqrt{\left\{ \frac{3720}{400} - \left( \frac{382}{400} \right)^2 \right\} \times (0.5)^2 - \left( \frac{1}{12} \text{ of } 0.25 \right)} = 1.4408$$

$$C. V. = \frac{\sigma}{M} \times 100 = \frac{1.4408 \times 100}{9.9775} = 14.44 \text{ per cent.}$$

The short cut methods give true values for the biometrical constants and not mere approximations to the results achieved by the long method.

In the preceding examples we were dealing with large samples in which the data were grouped. It sometimes happens, however, that the mean and the standard deviations have to be calculated from a few observations, in which case the calculation is made directly from the observed data. Thus in the following example of the yields of 25 plots of barley [Shaw and Bose, 1929] the calculations of these constants are shown below by two methods.

TABLE VII

*Calculation of the standard deviation, etc., by the Assumed Mean Method*

Assumed Mean = 330			
Yields in gm.	Deviations from assumed mean		Deviations squared
	—	+	
452 . . . . .	..	122	14884
352 . . . . .	..	22	484
450 . . . . .	..	120	14400
455 . . . . .	..	125	15625
372 . . . . .	..	42	1764
262 . . . . .	68	..	4624
275 . . . . .	55	..	3025
332 . . . . .	..	2	4

PLANT BREEDING AND AGRICULTURAL PROBLEM.

TABLE VII—*contd.*

*Calculation of the standard deviation, etc., by the Assumed Mean Method—contd.*

Assumed Mean = 330

Yields in grm.	Deviations from assumed mean		Deviations squared
	—	+	
252 . . . . .	78	..	6084
442 . . . . .	..	112	12544
204 . . . . .	126	..	15856
208 . . . . .	122	..	14884
280 . . . . .	50	..	2500
278 . . . . .	52	..	2704
201 . . . . .	129	..	16641
231 . . . . .	99	..	9801
313 . . . . .	17	..	289
181 . . . . .	149	..	22201
312 . . . . .	18	..	324
182 . . . . .	148	..	21904
472 . . . . .	..	142	20164
380 . . . . .	..	50	2500
316 . . . . .	14	..	196
358 . . . . .	..	28	784
308 . . . . .	22	..	484
TOTAL .	—1147	+765	204690

$$C \text{ (Correction factor)} = \frac{-1147 + 765}{25} = -15.28$$

$$\text{Mean} = 330 - 15.28 = 314.72$$

$$\begin{aligned} \sigma &= \sqrt{\frac{\sum d^2}{n} - \left(\frac{\sum d}{n}\right)^2} \\ &= \sqrt{\frac{204690}{25} - (-15.28)^2} \\ &= \sqrt{8187.60 - 233.48} \\ &= \sqrt{7954.12} = 89.19 \end{aligned}$$

$$E_m \text{ (Error of mean)} = \frac{0.6745 \times 89.19}{\sqrt{25}} = 12.03$$

TABLE VIII

*Calculation of standard deviation, etc., by the Yield Square Method, especially adapted for machine calculations*

Yield	(Yield) <sup>2</sup>
452	204304
352	123904
450	202500
455	207025
372	138384
262	68644
275	75625
332	110224
252	63504
442	195364
204	41616
208	43264
280	78400
278	77284
201	40401
231	53361
313	97969
181	32761
312	97344
182	33124
472	222784
380	144400
316	99856
358	128164
308	94864
<hr/>	<hr/>
TOTAL 7868	2675070
<hr/>	<hr/>

$$\text{Mean} = \frac{7868}{25} = 314.72$$

$$\begin{aligned}\sigma &= \sqrt{\frac{2675070}{25} - (314.72)^2} \\ &= \sqrt{107002.80 - 99048.6784} = \sqrt{7954.122} = 89.19\end{aligned}$$

$$E_m = \frac{0.6745 \times 89.19}{\sqrt{25}} = 12.03$$

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## CHAPTER IV

## PROBABILITY AND GOODNESS OF FIT

The normal curve represents the distribution of an infinite series of observations and is a perfectly smooth symmetrical curve in which the mean, median and mode coincide. In such a curve half of the total number of variates lie on the side above the mean and half on the side of the curve below the mean. With the median the quartiles divide the observations into four numerically equal groups and it follows, therefore, that one half of the total number of variates lie between the quartiles and one half lie beyond the range of the quartiles. In the normal perfectly symmetrical curve we may regard the mean value as representing the variate of most frequent occurrence, since mean and mode coincide in the normal curve, and variates of other values may be considered as deviations from the mean. The normal curve, therefore, represents the probability of occurrence of a variate of any value which is included in the distribution, those of values close to the mean being more frequent in occurrence than those of values approaching the limits of the distribution.

If in a normal frequency distribution we measure off on both sides of the mean a distance equal to the standard deviation we have an area, or range, that includes approximately 68 per cent of the total number of items in the distribution. The distance thus measured from both sides of the mean extends to points on a normal frequency curve where the curve changes from a concave to a convex surface, *i.e.*, at the points of inflexion.

In the case of the normal curve it can be shown mathematically that, if  $Q$  and  $Q'$  are the distances of the quartiles from the mean then

$$Q = Q' = 0.6745 \sigma,$$

or approximately

$$3 \text{ (distance of the quartile from mean)} = 2\sigma.$$

The relationship  $Q = 0.6745 \sigma$  is one which the student should memorize, its proof is outside the scope of this book.

In Figure 6, if  $M$  is the value of the mean and  $Q$  and  $Q'$  are the distances of the quartiles from the mean, then the two values  $M + Q'$  and  $M - Q$  will mark the limits between which fifty per cent of the variates will occur. It is, therefore, an even chance that any single variate picked at random from the sample will be of a value within these limits or outside them ; but

$$Q' = Q = 0.6745 \sigma$$

therefore, it is an even chance that any single variate picked at random will fall

within the values  $M \pm 0.6745 \sigma$ . The quantity  $0.6745 \sigma$  is called the *probable error of any variate*; this simply means that the odds are as 1 : 1 that any item in

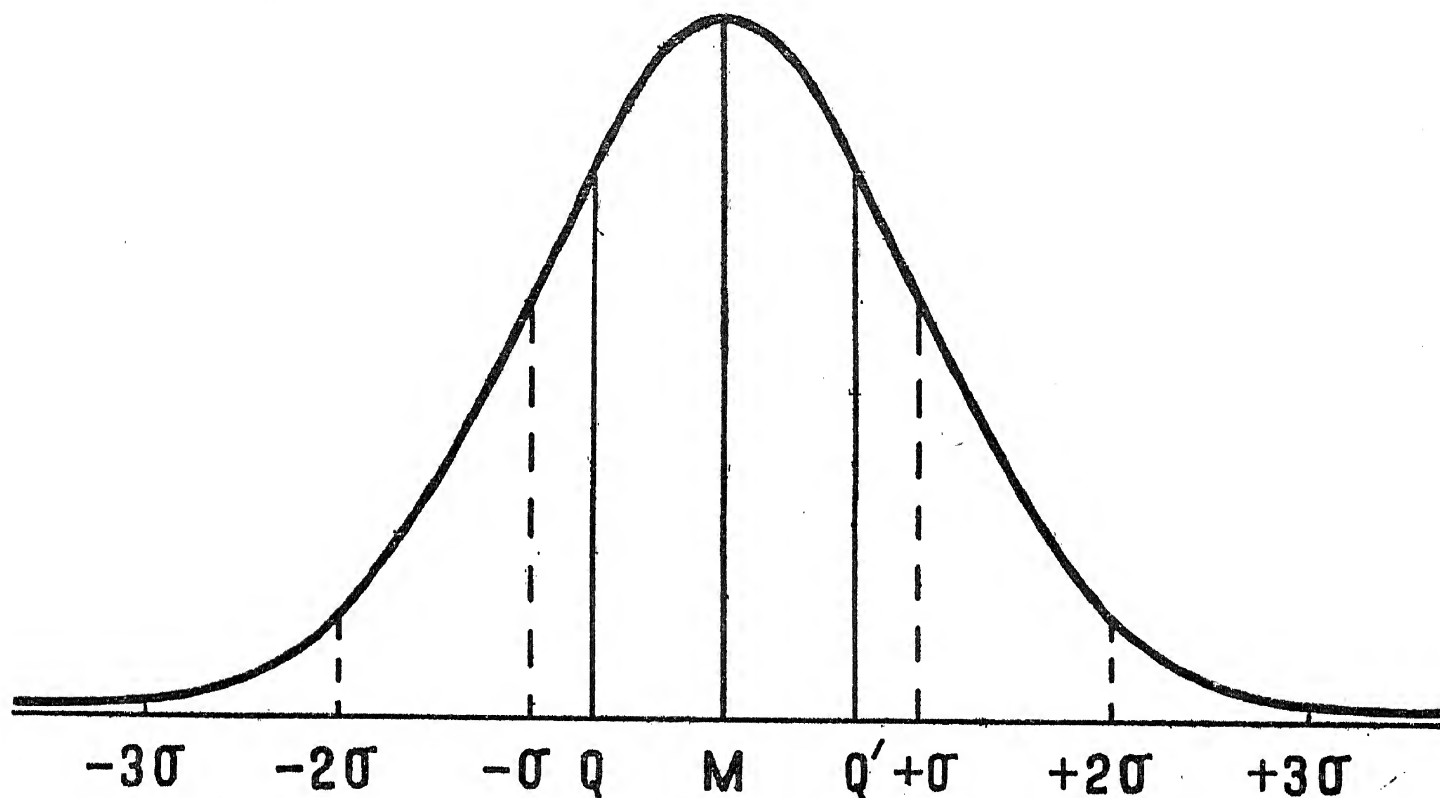


Fig. 6.—Normal curve showing relative position of mean, quartiles and various multiples of  $\sigma$ .

the distribution chosen at random will fall within the limits  $M \pm 0.6745 \sigma$ . In the normal curve the student will note that

- $M \pm (0.6745 \sigma)$  include 50 % of the variates
- $M \pm 2 (0.6745 \sigma)$  include 82.3 % of the variates
- $M \pm 3 (0.6745 \sigma)$  include 95.7 % of the variates
- $M \pm 4 (0.6745 \sigma)$  include 99.3 % of the variates
- $M \pm 5 (0.6745 \sigma)$  include 99.9 % of the variates,

similarly the values

- $M \pm \sigma$  include 68.3 % of the variates
- $M \pm 2 \sigma$  include 95.5 % of the variates
- $M \pm 3 \sigma$  include 99.7 % of the variates

Consideration of one of these values will make the significance of the foregoing clear. Let us take the value  $M \pm 2 (0.6745 \sigma)$ . Since 82.3 per cent of the variates are included within these limits 17.7 per cent will lie in the portions of the curve outside these limits, *i.e.*, towards the tails of the curve. The chances against the occurrence of a positive or negative deviation from the mean as large or larger than  $2 (0.6745 \sigma)$ , therefore, are as

$$82.3 : 17.7 \text{ or } 4.6 : 1$$

In other words, the odds are 4.6 : 1 against the random selection in a sample of a variate deviating from the mean by 2 ( $0.6745 \sigma$ ). The student should note carefully the difference between the odds against the occurrence of a *deviation* of a particular value and the odds against the occurrence of a *variate* of a particular size. Since we are considering a normal distribution of which 17.7 per cent of the items lie outside the limits  $M \pm 2$  ( $0.6745 \sigma$ ), it is obvious that 8.85 per cent of the variates will lie beyond the upper value,  $M + 2$  ( $0.6745 \sigma$ ) and 8.85 per cent will lie below the lower value,  $M - 2$  ( $0.6745 \sigma$ ); that is, each tail of the curve contains 8.85 per cent of the variates.

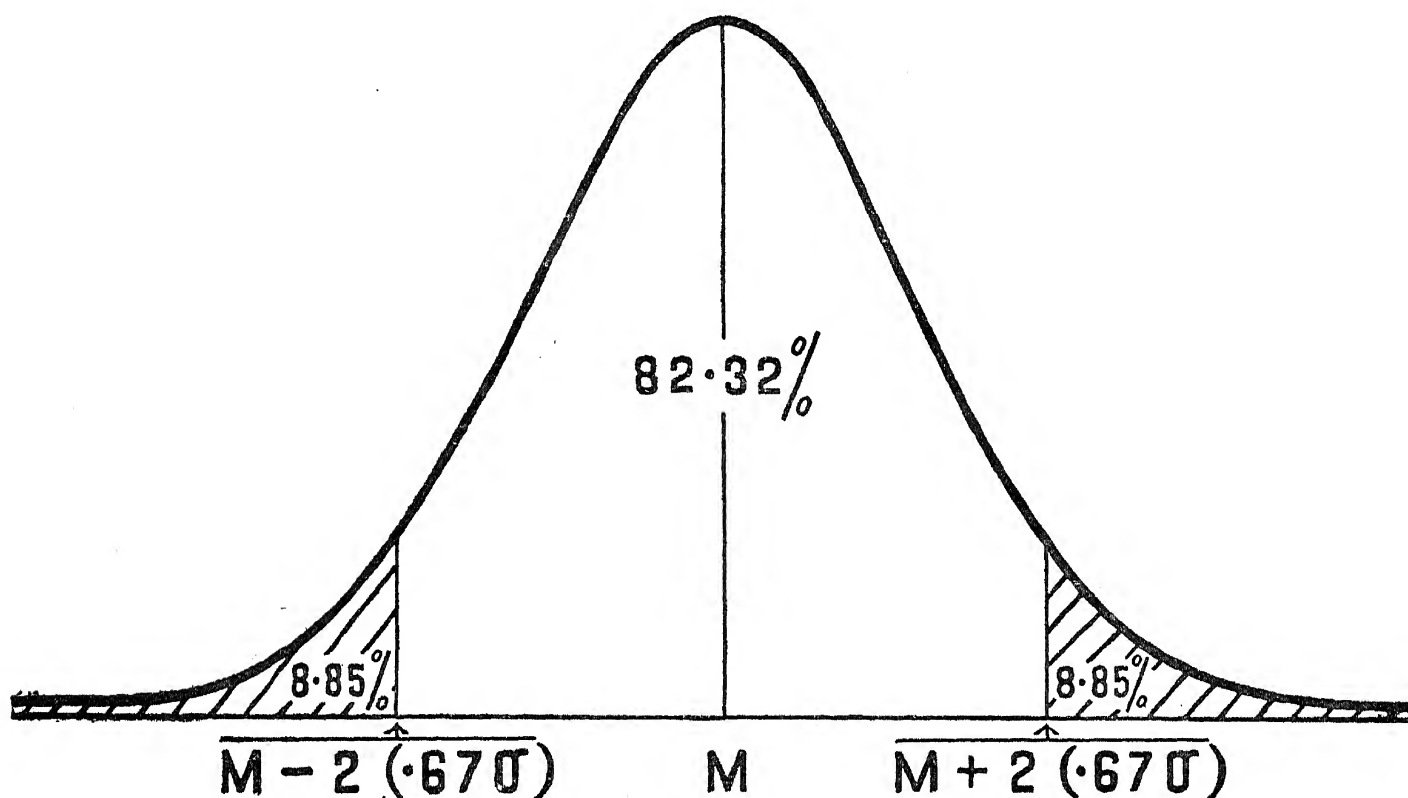


Fig. 7.—Distribution of variates within the limits  $M \pm 2$  ( $0.6745 \sigma$ ) in a normal curve.

Therefore, the odds against the occurrence of a variate as large as, or larger than,  $M + 2$  ( $0.6745 \sigma$ ) are as

$$(8.85 + 82.30) : 8.85$$

or

$$10.3 : 1$$

and similarly the odds against the occurrence of a variate as small as or smaller than  $M - 2$  ( $0.6745 \sigma$ ) are as

$$10.3 : 1.$$

Further consideration of the estimation of probability from the proportions of the normal curve will be deferred to the chapter on the Probability Integral.

## PROBABLE ERROR

In compiling frequencies and calculating means and other statistical constants the first essential is that the sample should be truly representative of the universe from which it is taken. We endeavour to secure this by taking as large a number of items as can be handled, and by taking them at random. No two samples, however, will exactly agree and, therefore, the means of a number of samples will vary very slightly, the extent of the variation depending upon the accuracy of the sampling. How can we estimate which of the values obtained for the mean or any other statistical constant from a number of different samples is the most accurate? An estimate of this is furnished by the quantity termed the *probable error* which can be readily calculated from the standard deviation of the constant. In practice, when we have determined a statistical constant, we put after it a value which represents the limits within which it may be expected that any subsequent determination from a similar sample will fall. This value is the probable error, an arbitrary term used to denote the amount which must be added to or subtracted from the observed value to obtain two limiting figures of which it may be said that it is an even chance that the true value lies within these limits.

The probable error of the mean of a sample is calculated by dividing the probable error of any variate by the square root of the population of the sample. Thus,

$$P.E._M = \frac{0.6745 \sigma}{\sqrt{n}} \quad \dots\dots\dots (7)$$

The student must distinguish carefully between the probable error of the mean and the probable error of any variate and note that the former is a function of the square root of the size of the sample; hence the importance of dealing with as large a sample as possible. The term, probable error, is a somewhat unfortunate one and is in no way to be taken as indicating the inaccuracy which is likely to occur in the course of an experiment; it is not the most probable mistake. The probable error has in modern statistics been largely superseded by the standard error. The formula for the standard error is the same as that for the probable error without the decimal fraction 0.6745. The standard error of the mean is, therefore,  $\frac{\sigma}{\sqrt{n}}$  and the relationship  $2 S.E. = 3 P.E.$  is approximately true.

The probable error of the standard deviation is given by a formula which is very similar to the above—

$$P.E. \sigma = \frac{0.6745 \sigma}{\sqrt{2n}} \quad \dots\dots\dots (8)$$

The fractions  $\frac{0.6745}{\sqrt{n}}$  and  $\frac{0.6745}{\sqrt{2n}}$  have been calculated for all values of  $n$  from 1 to 1,000 and are published in Pearson's Tables; this enormously facilitates the calculation of probable errors.



*Example 1.* In a sample of 400 heads of Pusa 12 wheat the mean number of grains per ear-head is 30.55 and the standard deviation is 7.117. From Pearson's Table V : 6.9992

$$\frac{0.6745}{\sqrt{400}} = 0.03372,$$

therefore,  $P.E._M = 0.03372 \times 6.9992 = 0.23601$ .

Similarly,  $\frac{0.6745}{\sqrt{400 \times 2}} = 0.02385,$

therefore,  $P.E. \sigma = 0.02385 \times 6.9992 = 0.16693$

From this example we see that the mean number of grains per ear-head is 30.55 and to this quantity we attach a probable error  $\pm 0.23601$ . It is usual to express these facts as

$$M = 30.55 \pm 0.23601$$

From this we understand that in any subsequent determination of the mean of this variable from a sample of the same size it is an even chance that the value obtained will lie between the limits 30.314 and 30.786. Similarly, the value of the standard deviation in the above example is

$$\sigma = 7.117 \pm 0.16693$$

The probable error of the coefficient of variation is given by the formula—

$$P.E. \text{ c.v.} = 0.6745 \ V \times \left\{ 1 + 2 \left( \frac{V}{100} \right)^2 \right\}^{\frac{1}{2}} / \sqrt{2n} \dots \dots (9)$$

where  $V$  is the coefficient of variation.

As already explained the fraction  $\frac{0.6745}{\sqrt{2n}}$  may be obtained from Pearson's tables for all values of  $n$  up to  $n = 1,000$ . The remainder of the expression may also be obtained from Pearson's tables for all values of  $V$  up to  $V = 50$ . The calculation of the probable error of the coefficient of variation, therefore, simply involves the product of two numbers which can be obtained from these tables. The long and complicated term

$$V \times \left\{ 1 + 2 \left( \frac{V}{100} \right)^2 \right\}^{\frac{1}{2}}$$

is referred to in Pearson's Table VI by the symbol  $\psi$ .

*Example 2.* The coefficient of variation of the number of grains per ear-head in Pusa 12 wheat is

$$V = \frac{\sigma}{M} \times 100$$

$$\therefore V = \frac{6.9992}{30.5500} \times 100$$

$$= 22.911$$

For the calculation of the probable error we find from the Tables that

$$\frac{0.6745}{\sqrt{400 \times 2}} = 0.02385$$

and when  $V = 22.911$ , then  $\psi = 24.0836$ ,

therefore  $P.E. \text{ C.V.} = 0.02385 \times 24.0836 = 0.5744$ ,

i.e.,  $C.V. = 22.911 \pm 0.5744$

*Probable error of a difference.*—Probable errors not only indicate the degree of confidence which may be placed in a result but are also useful for estimating the significance of a difference between two similar results. The probable error of the difference of two results depends on the mathematical theory of least squares and is the square root of the sum of the squares of the probable errors of the two results. Thus

$$E_d = \sqrt{E_1^2 + E_2^2} \dots \dots \dots (10)$$

where  $E_1$  and  $E_2$  are the two probable errors of two results and  $E_d$  is the probable error of the difference of these two results.

*Example 3.* Two determinations of the mean lengths of ear-head in Pusa 4 wheat, from similar samples, gave

$$M = 8.95 \pm 0.04 \text{ cm.}$$

$$\text{and } M = \underline{7.88} \pm 0.04 \text{ cm.}$$

the difference between these two means is 1.07 cm.

$$\text{and } E_d = \sqrt{(0.04)^2 + (0.04)^2} = 0.057,$$

so that the difference is  $1.07 \pm 0.057$  cm. and we see that the difference is approximately 18 times its probable error.

The probable error is a function of  $\sigma$ , therefore, 18 times the probable error can be expressed as a multiple of  $\sigma$  and we have seen that when a deviation is expressed as a multiple of  $\sigma$  we can estimate the chances of its occurrence, therefore, it is also possible to estimate the probability of a deviation which is expressed in terms of the probable error. Statisticians have adopted a standard that deviations of three or more times the probable error are significant of a real difference between the samples, such as would not be likely to occur by the operation of chance alone. In example 3 the observed deviation of 18 times the probable error is certainly significant of a real difference between the two samples, such as would be unlikely from

the results of chance errors. A table showing the probability of occurrence of deviations of different magnitudes relative to the probable error is given below :—

TABLE IX

*Probability of occurrence of statistical deviations of different magnitudes relative to the probable error*

Deviation divided by probable error	Probable occur- rence of a deviation as great as or greater than the expected one, expressed as a percentage	Odds against the occurrence of a deviation as great as or greater than the expected one	Deviation divided by probable error	Probable occur- rence of a deviation as great as or greater than the expected one, expressed as a percentage	Odds against the occurrence of a deviation as great as or greater than the expected one
1.0	50.00	1.00 : 1	3.1	3.65	26.40 : 1
1.1	45.81	1.18 : 1	3.2	3.09	31.36 : 1
1.2	41.83	1.39 : 1	3.3	2.60	37.46 : 1
1.3	38.06	1.63 : 1	3.4	2.18	44.87 : 1
1.4	34.50	1.90 : 1	3.5	1.82	53.95 : 1
1.5	31.17	2.21 : 1	3.6	1.52	64.79 : 1
1.6	28.05	2.57 : 1	3.7	1.26	78.37 : 1
1.7	25.15	2.98 : 1	3.8	1.04	95.15 : 1
1.8	22.47	3.45 : 1	3.9	0.853	116.23 : 1
1.9	20.00	4.00 : 1	4.0	0.698	142.26 : 1
2.0	17.73	4.64 : 1	4.1	0.569	174.75 : 1
2.1	15.67	5.38 : 1	4.2	0.461	215.92 : 1
2.2	13.78	6.26 : 1	4.3	0.373	267.10 : 1
2.3	12.08	7.28 : 1	4.4	0.300	332.33 : 1
2.4	10.55	8.48 : 1	4.5	0.240	415.67 : 1
2.5	9.18	9.89 : 1	4.6	0.192	519.83 : 1
2.6	7.95	11.58 : 1	4.7	0.152	656.89 : 1
2.7	6.86	13.58 : 1	4.8	0.121	825.45 : 1
2.8	5.90	15.95 : 1	4.9	0.095	1,051.63 : 1
2.9	5.05	18.80 : 1	5.0	0.074	1,350.35 : 1
3.0	4.30	22.26 : 1	6.0	0.0052	19,230.00 : 1

Thus from the above table we see that when

$$\frac{\text{Dev.}}{P.E.} = 4,$$

the probable occurrence in a hundred trials of a deviation as large as or larger than that observed is 0.698 and therefore the odds against the occurrence of such a deviation being due to chance alone are 142 : 1. *Then*

If we are confronted by a number of means and their probable errors ~~than~~ the probable error of the average of these means is given by the formula—

$$P.E. \text{ of an average of means} = \frac{1}{N} \sqrt{a^2 + b^2 + c^2 + \dots + n^2} \dots \dots \dots (11)$$

where  $N$  = the number of separate means and  $a, b, c, \dots, n$  represent the separate probable errors.

*Example 4.* The average length of ears of Pusa 4 wheat during the period 1925-26 to 1931-32 was :—

Year	Mean length of ear in cms.
1925-26 . . . . .	8.14 $\pm$ 0.04
1926-27 . . . . .	8.19 $\pm$ 0.03
1927-28 . . . . .	8.65 $\pm$ 0.03
1928-29 . . . . .	8.33 $\pm$ 0.03
1929-30 . . . . .	7.83 $\pm$ 0.03
1930-31 . . . . .	8.95 $\pm$ 0.04
1931-32 . . . . .	7.88 $\pm$ 0.04
Average of 7 years = 8.28	

*P.E. of the average*

$$= \frac{1}{7} \sqrt{(0.04)^2 + (0.03)^2 + (0.03)^2 + (0.03)^2 + (0.03)^2 + (0.04)^2 + (0.04)^2}$$

$$= 0.0131$$

That is the average length of Pusa 4 wheat in these 7 years was  $8.28 \pm 0.0131$  cms.

#### PROBABLE ERROR OF AN OBSERVED PROBABILITY

Two or more events are mutually exclusive if the occurrence of one excludes the occurrence of the other. Thus, if a coin is tossed upon a flat table it will fall in one of two ways—either head up or tail up and both ways are equally likely and with a single coin both ways cannot occur at one throw. The two events are, therefore, mutually exclusive, and the probability of each event is 0.5, since there are only two possibilities, heads or tails, and one of them must occur. If  $p$  is the



In this case the deviation is less than the probable error and is such as a chance error of the experiment.

A word of caution is necessary in the use of these formulae. They are valid when neither  $p$  nor  $q$  is very small, and in more complicated Mendels such as 63 : 1, one of these terms would be represented by  $\frac{1}{64}$ th, in which case it is useless to apply these formulae unless  $n$  is very large.

Moreover, it is theoretically unjustifiable to test the validity of a given determination of the probable error of one or all of its individual groups; the random deviations of the class frequencies are not independent but are correlated.

#### GOODNESS OF FIT

A criterion of the goodness of fit of observations to expectations, which is not open to the above objections, is the application of what is known as the Chi-square test.  $\chi^2$  is calculated from the following formula :—

$$\chi^2 = \sum \frac{(O - C)^2}{C} \dots\dots\dots (15) \quad \chi^2 = \sum \frac{(O - C)^2}{C}$$

where  $O$  = the observed frequency of a class,

$C$  = the calculated frequency of a class,

and  $\sum$  indicates summation of the term for all classes in the ratio.

This method has the advantage that it gives a measure of the goodness of fit of the ratio as a whole and allows of the class frequencies which deviate most from expectation being determined at a glance from the value of  $\frac{(O - C)^2}{C}$ .

The distribution of  $\chi^2$  corresponding to the number ( $n$ ) of classes in the distribution, was worked out by Pearson and a table was constructed (Elderton) showing the probability of an observed value of  $\chi^2$  being given by a random sample from a hypothetical population. That is to say, from the value of  $\chi^2$  we can deduce the probability that random sampling would lead to as large a deviation or a larger deviation between theory and observation. Thus, with 18 classes and a calculated value of  $\chi^2 = 10$ , we find from the table that  $P = 0.903$  and conclude that, in 90 cases out of hundred trials, the chance errors of random sampling would give a deviation as large as or larger than that observed; we may, therefore, say that the agreement between observation and theory in such a case is excellent.

*Example 6.* In the  $F_2$  population of a cross between type 22 linseed which has a blue petal and type 12 which has a white petal, the following frequencies were observed [Shaw, Khan and Alam, 1931]:—

Phenotype	FREQUENCY		$O - C$	$\frac{(O - C)^2}{C}$
	Observed	Expected on 9 : 3 : 3 : 1		
Blue like $F_1$ . . . .	205	217.6875	-12.6875	0.7395
Blue like type 22 . . .	80	72.5625	7.4375	0.7623
White like type 12 . . .	76	72.5625	3.4375	0.1628
White . . . . .	26	24.1875	1.8125	0.1358
				$\chi^2 = 1.8004$

Referring to Pearson's Table XII we find that when  $n = 4$  and  $\chi^2 = 1$ ,  $P$  is 0.8013 and when  $\chi^2 = 2$ ,  $P$  is 0.5724, by interpolation we find the value of  $P$  corresponding to  $\chi^2 = 1.80$  is 0.6182. We conclude, therefore, that in about 62 cases in a hundred trials, chance errors of random sampling would give deviations as large as those observed and that the fit of observation to theory is satisfactory.

Fisher, in his Table III, has published the  $\chi^2$  table in a slightly different form in which values of  $P$  corresponding to values of  $\chi^2$  smaller than one are tabulated and in which values of  $\chi^2$  corresponding to specially selected values of  $P$  are given. Fisher's Table is entered with  $n$  equal to one less than the number of classes in the distribution, this value is known as the degrees of freedom and is explained in a later chapter.

*Example 7.* In the  $F_2$  population of a cross between type 3 chilli which is purple in colour and type 29 which is green, the following frequencies were observed [Deshpande, 1933]:—

Phenotype	FREQUENCY		$O - C$	$\frac{(O - C)^2}{C}$
	Observed	Expected on 1 : 3 : 8 : 4		
Purple, deep . . . .	65	68.75	-3.75	0.2045
Purple, medium . . .	203	206.25	-3.25	0.0512
Purple, light . . . .	563	550.00	+13.00	0.3073
Green . . . . .	269	275.00	-6.00	0.1309
				$\chi^2 = 0.6939$

From Fisher's Tables, entering with  $n = 3$ , we find that this value of  $\chi^2$  lies between  $P = 0.90$  and  $P = 0.80$  and we, therefore, conclude that the fit is good.

#### THE PROBABILITY THAT TWO GIVEN SAMPLES BELONG TO THE SAME UNIVERSE

In many cases of statistical investigation, we have to deal with samples drawn from different places and it is necessary to decide whether they belong to the same universe or not. Suppose that we have two samples of spores of the same species of fungus from two different localities. The frequency distribution of length in each sample may show differences between the two samples in respect to this character and it is necessary to be able to decide whether the observed difference is of such an order as to preclude the two samples belonging to the same universe.

A fair idea of the nature of the discrepancy between two given frequency distributions can be had by comparing the means, the standard deviations and other statistical constants of the two samples in question. Let  $m_1, \sigma_1, n_1$  and  $m_2, \sigma_2, n_2$  be the means, the standard deviations and the sizes of the two samples. If the differences between the means or the standard deviations are not significant, we say that the two samples belong to the same population, and if all the other statistical constants such as coefficient of variability, etc., also do not show any significant difference then the chance of two samples belonging to the same population is greater still.

It is known that the mean, the standard deviation and other statistical constants of a frequency distribution summarise the most important properties of the distribution. But an index which takes into account the differences in frequencies of two samples for the same interval is bound to afford a better estimate for a comparison between them. In the  $\chi^2$  test our object is to see whether a frequency distribution differs widely from a theoretical one. The expected values of the different class intervals are calculated and an index based upon the differences of the expected and actual frequencies for the respective class intervals enables us to determine the probability that the given distribution can be represented by a known theoretical distribution. The use of the  $\chi^2$  table has been fully explained in the previous section of this chapter.

By using a principle similar to the one explained above, Pearson [*Biometrika*, Vol. VIII, 1911] showed that the probability of two samples of sizes  $N$  and  $N'$  belonging to the same population, can be determined from " $\chi^2$ " Table by evaluating

$$\chi^2 = \frac{NN'}{N + N'} \sum \left\{ \frac{f_s}{N} - \frac{f'_s}{N'} \right\}^2 / p_s, \text{ where } p_s = \frac{f_s + f'_s}{N + N'}, f_1, f_2, \dots, f_p, \dots, f_s$$

and  $f'_1, f'_2, \dots, f'_p, \dots, f'_s$  are the frequencies of the samples for corresponding class intervals. But later he finds [*Biometrika*, Vol. XXIV, 1932] that  $p_s$  is the probability that any selection will fall in the  $s$ th class frequency as indicated by the two samples together. If the samples are considerable in size, the assumed value of  $p_s$  is correct. The biologist is generally dealing with small samples and



Pearson has found [1932] that the best way to estimate the value of the probability that the two samples belong to the same universe is by evaluating

$$\chi^2 = \frac{NN'}{N + N'} \left[ \sum \left( \frac{f_s}{N} - \frac{f'_s}{N'} \right)^2 \right] \dots\dots (15A)$$

where  $N$  and  $N' =$  the size of the two samples and  $f_s$  and  $f'_s =$  the frequencies of the two samples for the corresponding class intervals. Knowing the value of  $\chi^2$  for any two particular distributions, the maximum probability of the two samples belonging to the same population can be determined from  $\chi^2$  table.

Table X gives the frequency distribution of the length of each of the two hundred spores of Peshawar and Karnal strains of *Tilletia indica*.

TABLE X

*Frequency distributions of the length of spores*

Spore length $\mu$	FREQUENCY OF <i>T. INDICA</i>	
	Karnal strain $f_s$	Peshawar strain $f'_s$
25	2	0
28	4	3
31	20	4
34	24	81
37	66	91
40	43	3
43	28	14
46	5	4
49	6	0
52	0	0
55	2	0
TOTAL	200 $N$	200 $N'$

The means, the standard deviations and the coefficient of variation of the measurements of the two strains of spores are given in Table XI.

TABLE XI

*Biometrical constants of the measurements of spores*

Constants	LENGTH IN $\mu$		RATIO OF DIFFERENCE TO S. ERROR OF DIFFERENCE
	Peshawar strain	Karnal strain	
Means	36.175	37.990	4.6105
Standard deviation	2.9024	4.7508	6.6394
Coefficient of variation	8.0232	12.5054	5.9564

A comparison of the means, the standard deviations and variations of the two strains in respect to length shows that the differences are statistically significant and hence the probability that they belong to the same group or population is small.

Now let us apply the method developed by Karl Pearson to the data. For this we have to evaluate the expression

$$\chi^2 = \frac{NN'}{N+N'} \left[ \sum \left( \frac{f_n}{N} - \frac{f'_n}{N'} \right)^2 \right]$$

In this particular case  $N = N' = 200$

Hence the expression reduces to  $\frac{1}{N+N'} \left[ \sum \{ f \sim f' \}^2 \right]$

The data are shown in detail in Table XII.

TABLE XII

*Frequency distributions of Karnal and Peshawar strains of Tilletia indica*

Spore length $\mu$	FREQUENCY OF <i>T. INDICA</i>		$ f_n - f'_n $
	Karnal strain $f_n$	Peshawar strain $f'_n$	
1	2	3	4
25	2	0	2
28	4	3	1
31	20	4	16
34	24	81	57
37	66	91	25
40	43	3	40
43	28	14	14
46	5	4	1
49	6	0	6
52	0	0	0
55	2	0	2

Algebraic sum of column 4 = 164

$$\chi^2 = \frac{(164)^2}{400} = 67.24$$

and the probability for  $\chi^2 = 67.24$ ,  $n' = 11$  is .00000 showing that the two strains belong to entirely different populations.

The method indicated above holds good when we are dealing only with one single character, *viz.*, length in this case. But when a sample has got a number of characters such as length, breadth, height, etc., a rough way of testing the universality of the two samples is to evaluate the expression

$$\frac{NN'}{N+N'} \left[ \sum \left\{ \frac{f_s}{N} \sim \frac{f'_s}{N'} \right\} \right]^2$$

as mentioned above, and if for every one of the characters the probability of the two samples being taken from the same population is high, then we can safely assert that the samples belong to one and the same universe. But this need not be the case in the statistical problems that frequently occur in biological work. The probability for two of the characters might be high and it might be low for a third character. For such cases Karl Pearson has developed another index known as the Coefficient of Racial Likeness based upon all the characters in consideration [*vide Biometrika*, Vol. XVIII]. This has been further developed by Professor P. C. Mahalanobis in his article on Tests and Measures of Group Divergence, published in the *Journal and Proceedings of the Asiatic Society of Bengal*, Vol. XXVI, 1930, No. 4. The student interested in further study of such problems is referred to the above mentioned original articles.

### LINKAGE VALUES

In Mendelian studies it frequently happens that the inheritance of two pairs of characters does not conform to the normal expectation but that certain combination of characters occur more frequently than should be the case according to the ordinary expectations of independent segregation. In such cases characters are said to be linked together. Although a description of the methods and significance of linkage in inheritance is outside the scope of this book, yet since various mathematical methods of calculating the strength or intensity of linkage from observed phenotypic frequencies in  $F_2$  have been evolved, a few examples of the calculation of linkage values are included here.

The intensity of linkage between two characters can obviously be estimated from the ratio between the number of occasions on which they occur together to the number of occasions on which they occur separately, or in other words, the probability of the two characters occurring separately, expressed as a percentage, will be an estimate of the linkage value. The separation of two characters which are usually linked together depends upon the crossing over from one chromosome to another of a gene carrying one of the characters, hence the percentage probability of the separation of two characters is generally called the cross-over value.

*Example 8.* A few methods of calculating linkage values may be illustrated from an observed case of linkage in linseed. Linseed type 11 has a deep lilac petal and a deep purple stigma and linseed type 121 has a lilac petal and a white stigma

[Shaw *et. al.*, 1931]. In  $F_1$  the type 121 phenotype was dominant and in  $F_2$  the following frequencies were observed :—

	LILAC PETAL		DEEP LILAC PETAL	
	White stigma	Purple stigma	White stigma	Purple stigma
Observed	$AB$ 357	$Ab$ 37	$aB$ 33	$ab$ 94
Expected on 9 : 3 : 3 : 1	293.04	97.68	97.68	32.56

From an inspection of these frequencies it is obvious that the parental combinations occur more frequently than should be the case on a 9 : 3 : 3 : 1 ratio, but when either the petal or the stigma characters are taken separately a good 3 : 1 fit is obtained, *e.g.*, white stigmas 390, purple stigmas 131.

*Additive method.* This method of calculating the linkage from observed phenotypic frequencies in  $F_2$  is, as its name implies, based upon the summation of class frequencies. A simple formula is that of Emerson's—

$$p^2 = \frac{E - M}{n} \dots\dots\dots(16)$$

where  $E$  = Sum of the frequencies of the two end classes (the double dominant and the double recessive)

$M$  = Sum of the frequencies of the two middle classes (single dominants)

$n$  = Total population

and  $1 - p$  = the percentage of crossing-over.

Applying this formula to the data of linseed above, we get

$$p^2 = \frac{(357 + 94) - (33 + 37)}{521} = \frac{381}{521} = 0.73$$

$$p = \sqrt{0.73} = 0.854$$

$$1 - p = 1 - 0.854 = 0.146,$$

therefore, the cross-over value is 14.6 per cent, which represents the chance of occurrence of the gametes in which the linkage has broken down. The gametic ratio, therefore, is—

$AB$	$Ab$	$aB$	$ab$
42.7	7.3	7.3	42.7

or approximately      6                      1                      1                      6

*Product ratio method.* This method, as its name implies, depends upon the ratio of the product of the two end classes to that of the two middle classes.

$$P = \frac{ad}{bc} \dots\dots\dots(17)$$

where  $a$  = the frequency of the double dominant class

$d$  = the frequency of the double recessive class

and  $b$  and  $c$  = the frequency of the single dominant classes

$$p^2 = \frac{P + 1 - \sqrt{3P + 1}}{P - 1} \dots\dots\dots(18)$$

Applying these formulae to the data from linseed, we get

$$P = \frac{357 \times 94}{33 \times 37} = 27.48$$

$$p^2 = \frac{27.48 + 1 - \sqrt{(3 \times 27.48) + 1}}{27.48 - 1}$$

$$= 0.728.$$

The value of  $p^2$  agrees with that obtained by the additive method.

Another method of calculating linkage values is based upon the square root of the proportional frequency of the double recessive. Since the double recessive is, in the normal dihybrid ratio, represented by only one individual it is obvious that the square root of the proportional frequency, expressed as a decimal fraction, of the double recessive, will give directly the probability of the occurrence of the double recessive gamete. Table XIII shows how the data are arranged for calculation by this method :—

TABLE XIII

*Computation of linkage value from  $F_2$  data of flower colour in linseed type  
121  $\times$  type 11*

Phenotypes	$F_2$ fre- quency	Observed propor- tions	9 : 3 : 3 : 1 propor- tions	Deviation	Adjusted propor- tions	Calculated fre- quency
1	2	3	4	5	6	7
Lilac petal and white stigma.	357	0.6852	0.5625	0.1227	0.6828	355.74
Lilac petal and purple stigma.	37	0.0710	0.1875	0.1165	0.0672	35.01
Deep lilac petal and white stigma.	33	0.0633	0.1875	0.1242	0.0672	35.01
Deep lilac petal and purple stigma.	94	0.1804	0.0625	0.1179	0.1828	95.24

Average deviation . 0.1203

The adjusted proportions in column 6 of the above table are obtained by adding the average deviation to the first and last terms and subtracting it from the two

middle terms in column 4 which contains the normal proportions. The of occurrence of the double recessive gamete will be the square root of proportionate occurrence of the double recessive phenotype. In this  $c = 0.42755$ , therefore, the frequency of the double recessive gamete is 42.7 per cent, a result which agrees with that previously calculated. The calculated frequencies in column 7 are obtained by multiplying the observed frequencies in the various classes by their respective adjusted proportions and dividing this product by the observed proportions. Thus, in the last phenotypic class, the calculated frequency is  $\frac{94 \times 0.1828}{0.1804}$  or 95.24.

A full description of the methods of computing linkage values has recently been published by Alam [1929].

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## CHAPTER V

## PROBABILITY INTEGRAL

If we consider the diagram (fig. 4) of a normal curve, we see that the upper quartile divides the curve into two areas, 75 per cent of the total area of the curve being on the left of the quartile and 25 per cent on its right. If  $M$  is the value of the mean and  $x$  represents the deviation of the upper quartile from the mean, then the value of the ~~ordinate~~ at the upper quartile is  $M + x$ . All items in the distribution of a size as large as or larger than  $M + x$  will occur in the portion of the curve included in the smaller area, which is 25 per cent of the total area enclosed by the curve. Since this smaller area is to the remaining area of the curve in the proportion of 25 : 75, it is clear that the odds against the occurrence of an item as large as  $M + x$  or larger will be as 3 : 1. The student should particularly note that the odds are quoted *against* the occurrence of a particular value, the *chance* of occurrence of a value of  $M + x$ , or larger, is of course, as 1 : 3.

In this particular example the deviation from the mean,  $x$ , is equal to the distance of the upper quartile from the mean and we have already shown that a deviation of this size is equal to  $0.6745 \sigma$ . It follows, therefore, that in this case

$$x = 0.6745 \sigma$$

$$\text{and } \frac{x}{\sigma} = 0.6745.$$

We infer, therefore, that in a frequency distribution which follows the normal curve, when the deviation,  $x$ , from the mean divided by  $\sigma$  gives a quotient of 0.6745 that the ordinate erected at  $x$  will divide the area of the curve in the ratio of 3 : 1. It is possible to calculate the proportions into which the area of the normal curve will be divided by ordinates erected at any deviations from the mean, these deviations being expressed in terms of  $\sigma$ . In other words, the ratio  $\frac{x}{\sigma}$  will for normal distributions bear a definite relation to the proportions into which the curve is divided by the ordinate erected at the deviations  $M \pm x$ . Mathematicians have, for the normal curve, constructed tables giving for all values of  $\frac{x}{\sigma}$  the proportionate areas cut off by the ordinates at those points; such tables are called tables of the *probability integrals*. Hence, for any value of  $\frac{x}{\sigma}$  where  $x$  is the deviation from the mean, we can estimate the chances of occurrence of a deviation of this size or greater. Pearson's Table II (Vol. I) gives all values of  $\frac{x}{\sigma}$  from 0 to 6.00 and the ratio of the corresponding areas into which the curve is divided. The larger area of the two areas into which the curve is divided by the ordinate  $\frac{x}{\sigma}$  is given in the column  $\frac{1}{2}(1 + \alpha)$ , and the smaller area is determined by subtracting this from 1. The probability integral may be defined as the proportion of the area lying under the curve between the lower extremity and the ordinate at any given value.

*Example 9.* The mean height in a sample of 1,000 maize plants is 64.64 inches with a standard deviation of 2.7. What are the chances of the occurrence of a plant 70.04 inches or more ?

$$x = 70.04 - 64.64 = 5.4 \text{ inches.}$$

$$\frac{x}{\sigma} = \frac{5.4}{2.7} = 2$$

From Pearson's Table II, we find that when the value  $\frac{x}{\sigma}$  is 2, the area of the curve cut off by the ordinate at the deviation  $+5.4$  from the mean is 0.9772 of the whole area of the curve. The curve, therefore, is divided into two parts in the ratio of 0.9772 : (1-0.9772) or 97.72 : 2.28. The chances of occurrence of a plant of 70.04 inches or larger are about two and a quarter in a hundred. In other words, the odds against the occurrence of a variate 70.04 inches or larger are given by the ratio

$$97.72 : 2.28$$

or approximately 43 : 1 *against* the occurrence.

If we are considering the chances of occurrence of individuals deviating from the mean by 5.4 inches or more, the odds will be different, since in this case we are dealing with deviations both above and below the mean ; that is to say, we are considering the chances of occurrence of individuals of 70.04 inches or more and of 59.24 inches or less. Figure 8 will make this clear ; the deviation  $x$ , 5.4 inches, has

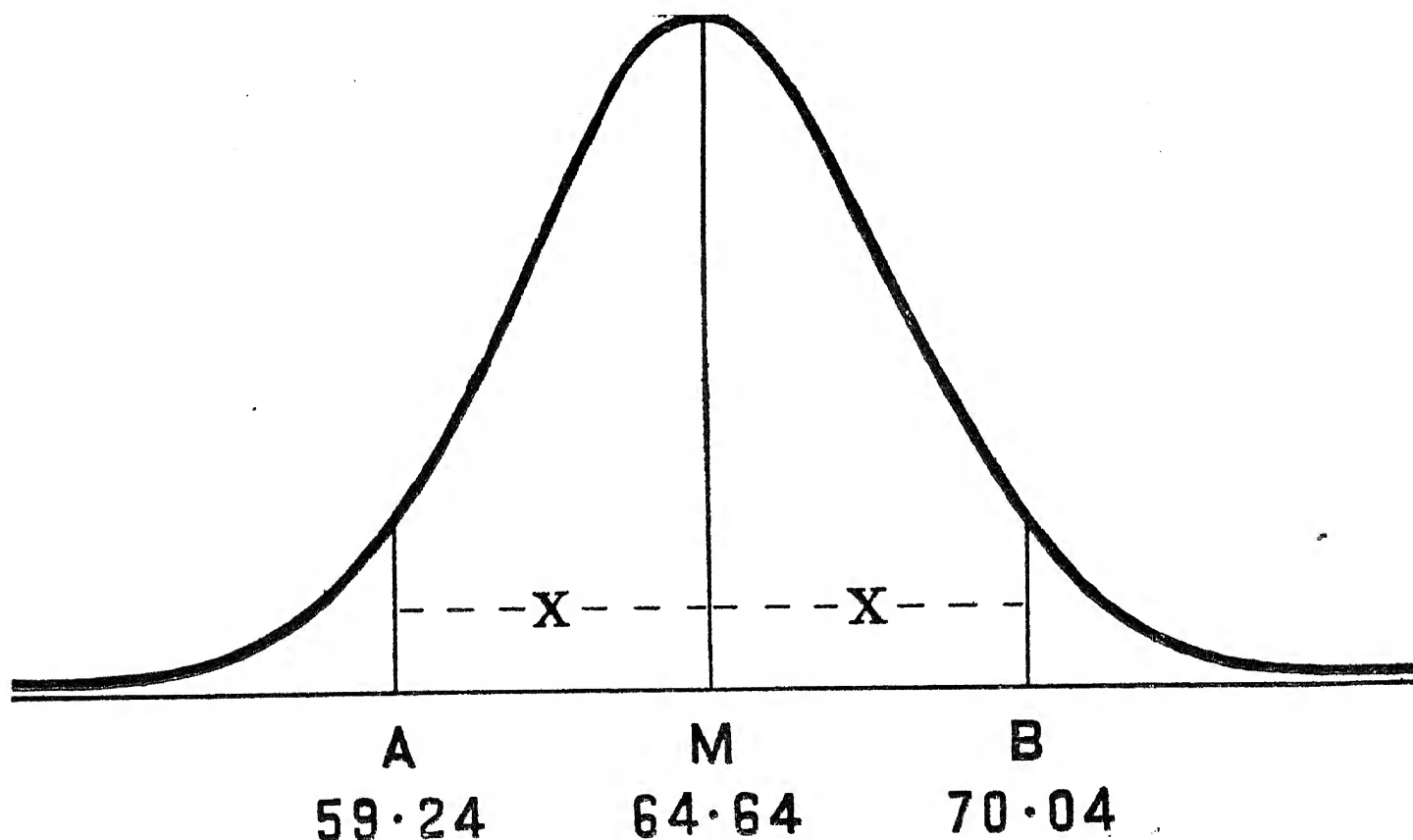


Fig. 8.—Probability integral



a positive value on the right hand side of the mean and a negative value on the left hand side of the mean, the ordinates erected at  $B$  and  $A$  marking respectively the positive and negative values of the deviation.

Now we have seen that the ordinate at  $B$  divides the area of the curve into two areas in the ratios of 0.9772 : 0.0228 and similarly the ordinate at  $A$  will also divide the *total* area of the curve in these same proportions. It follows, therefore, that each tail of the curve, that is to say the smaller areas lying respectively to the right and left of the ordinates at  $B$  and  $A$ , is 0.0228 of the whole area, and the proportion of the total area between the ordinates at  $A$  and  $B$  is  $1 - (2 \times 0.0228)$  or 0.9544. The odds against the occurrence, therefore, of a variate differing from the mean by ~~5~~ 5.4 inches <sup>or more</sup> will be the ratio of the area of the curve between the ordinates at  $A$  and  $B$  to the sum of the areas of the two tails, that is to say as 0.9544 : 0.0456 or approximately as 21 : 1. The ordinates at  $A$  and  $B$  cut off two tails which are approximately 5 per cent of the total area of the curve.

The student must distinguish carefully between problems which involve the area of one tail of the curve relative to the remainder and problems which involve the sum of the areas of both tails relative to the central area.

Table XIV shows for a few values of  $\frac{x}{\sigma}$  the relative proportions of the areas into which the curve is divided by the ordinate at  $x$  and the odds against the occurrence of a deviation of  $x$ . Thus, when  $\frac{x}{\sigma} = 1$ , the sum of the areas in both tails of the curve is 0.31732 and the area lying between the tails is 0.68268 and the odds against the occurrence of a deviation of  $x$  are as 68268 : 31732 or approximately as 68 : 32 which is about 2.12 : 1.

TABLE XIV

*Showing the greater portion of the area of a normal curve of errors to one side of an ordinate at the abscissa  $\frac{x}{\sigma}$  together with its relation to  $P$  (value of two tails)*

$\frac{x}{\sigma}$	Greater fraction of area	Area in one tail	Area in two tails or $P$	Odds against occurrence of <del>at</del> <i>bigg</i> deviate <i>than</i> $\frac{x}{\sigma}$	
0	0.50000	0.50000	1.00000	0 : 100	..
0.1	0.53983	0.46017	0.92034	8 : 92	0.087 : 1
0.2	0.57926	0.42074	0.84148	16 : 84	0.190 : 1
0.3	0.61791	0.38209	0.76418	24 : 76	0.316 : 1
0.4	0.65542	0.34458	0.68916	31 : 69	0.449 : 1
0.5	0.69146	0.30854	0.61708	38 : 62	0.613 : 1

TABLE XIV—*contd*

$\frac{x}{\sigma}$	Greater fraction of area	Area in one tail	Area in two tails or $P$	Odds against occurrence of $a \pm$ deviate	
0.6 .	0.72575	0.27425	0.54850	45 : 55	0.818 : 1
0.7 .	0.75804	0.24196	0.48392	52 : 48	1.083 : 1
0.8 .	0.78814	0.21186	0.42372	58 : 42	1.381 : 1
0.9 .	0.81594	0.18406	0.36812	63 : 37	1.703 : 1
1.0 .	0.84134	0.15866	0.31732	68 : 32	2.12 : 1
1.1 .	0.86433	0.13567	0.27134	73 : 27	2.71 : 1
1.2 .	0.88493	0.11507	0.23014	77 : 23	3.35 : 1
1.3 .	0.90320	0.09680	0.19360	81 : 19	4.26 : 1
1.4 .	0.91924	0.08076	0.16152	84 : 16	5.25 : 1
1.5 .	0.93319	0.06681	0.13362	87 : 13	6.69 : 1
1.6 .	0.94520	0.05480	0.10960	89 : 11	8.09 : 1
1.7 .	0.95543	0.04457	0.08914	91 : 9	10.10 : 1
1.8 .	0.96407	0.03593	0.07186	93 : 7	13.28 : 1
1.9 .	0.97128	0.02872	0.05744	94 : 6	15.67 : 1
2.0 .	0.97725	0.02275	0.04550	95 : 5	19.0 : 1
2.1 .	0.98214	0.01786	0.03572	96 : 4	24.0 : 1
2.2 .	0.98610	0.01390	0.02780	97 : 3	32.33 : 1
2.3 .	0.98928	0.01072	0.02144	98 : 2	49.0 : 1
2.4 .	0.99180	0.00820	0.01640	98 : 2	49.0 : 1
2.5 .	0.99379	0.00621	0.01242	99 : 1	99.0 : 1

The following example will serve to familiarise the student with the use of the probability integral table.

*Example 10.* The mean length of ear-head in a sample of 400 ears of Pusa 12 wheat is 9.9775 cms. with a standard deviation of 1.4408 cms. What are the chances of the occurrence of :

- (1) a head of 12.1282 cms. or more,  
 (2) a head of 6.5364 cms. or less,  
 and (3) a head deviating from the mean by ~~2.58084~~ <sup>or more</sup> 2.58084 cms. <sup>(?)</sup> It will be seen that

$$(1) \frac{x}{\sigma} = \frac{2.1507}{1.4408} = 1.4927.$$

From table of probability integral  $\frac{1}{2} (1 + \alpha) = 0.93224$ .

Area in one tail = 0.06776.

Odds approximately 93 : 7 or 14 : 1 against.

$$(2) \frac{x}{\sigma} = \frac{3.4411}{1.4408} = 2.3883.$$

Area in one tail = 0.00846.

Odds approximately 992 : 8 or 121 : 1 against.

$$(3) \frac{x}{\sigma} = \frac{2.58084}{1.4408} = 1.7915$$

$$\frac{1}{2} (1 + \alpha) = 0.96335$$

Area in two tails = 0.07330

Area between the tails = 0.9267 or  $(1 - 0.07330)$

Odds approximately 927 : 73 or 12.7 : 1 against.

The probability integral is the fundamental basis for a large part of statistical work. The examples which we have dealt with up to now have been taken from samples which are relatively large and which closely approximate to a normal distribution. The special methods necessary for dealing with small samples in which the distribution may depart from the normal are dealt with in subsequent chapters.

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## CHAPTER VI

## SIGNIFICANCE OF DIFFERENCES BETWEEN MEANS

One of the most frequent problems in biological work is the comparison of the mean values of different samples. We may, for instance, determine the mean length of ear-heads in different varieties of wheat and attempt to estimate the significance of the differences between the various means, or a more common problem is the determination of the significance of the differences between the mean yields of different varieties. In any branch of experimental science it is usual to repeat a particular experiment several times and to form an estimate of the reliability of the result from a comparison of the amount of agreement between the repetitions; such an estimate may be furnished by the size of the difference between the largest and smallest determination relative to the size of the determinations themselves. This practice is sufficient in experimental work in physical and chemical sciences in which the conditions of the experiment are more rigidly under control than in biological work and in which, therefore, the fluctuations between the results of repetitions of the same experiment are generally insignificant. In experimental work in plant breeding and agriculture, the material of the experiment is a living plant subject to the uncontrolled fluctuations of soil and climate and the comparison of experimental results must be made by the application of rigid statistical tests based on representative samples.

It is theoretically possible to take an infinite number of samples of the same size from an infinite population, and to determine the mean of each sample; these means will differ slightly from each other provided  $n$  is sufficiently large. If the samples follow a normal distribution then these means will also be distributed normally about the average of all the means, and the standard deviation of this curve will represent the variability present in the universe. The larger the number of samples taken the more this curve, which is called the *sampling distribution of the mean*, will approximate to the normal smooth curve, and mathematicians have established that such

a curve has a standard deviation of  $\frac{\sigma}{\sqrt{n}}$ , where  $\sigma$  is the standard deviation of the

universe and  $n$  is the size of the sample. This means that if a quantity is normally distributed with standard deviation  $\sigma$ , then the means of samples containing  $n$

items are normally distributed with standard deviation  $\frac{\sigma}{\sqrt{n}}$ . This quantity  $\frac{\sigma}{\sqrt{n}}$

is called the STANDARD ERROR and can be easily determined for any sample of size  $n$  if the  $\sigma$  of the population is known. We have shown in our consideration of the probability integral that the probability of occurrence of a given deviation from

the mean can be estimated by the ratio  $\frac{\text{deviation}}{\text{standard deviation}}$  in quantities which

follow a normal distribution. Since  $\frac{\sigma}{\sqrt{n}}$  is the standard deviation of the sampling distribution of means, the significance of the difference between the mean of a sample and the true mean of the universe, which is represented by the average of the means of all samples, can be estimated by

$$\frac{\text{deviation}}{\frac{\sigma}{\sqrt{n}}}$$

In actual practice the true mean of the universe is a hypothetical quantity which we do not know and our comparison is always between the means of different samples. If we take a number of different samples from a normal population and take the differences between pairs of means then it can be shown mathematically that such differences also follow a normal distribution and that for any two means the standard error of the difference between them is given by

$$S. E._{d(1-2)} = \sqrt{(S. E._1)^2 + (S. E._2)^2} \dots\dots\dots(19)$$

The ratio of difference to the standard error of the difference,  $\frac{d}{S. E._d}$ , can then be used to determine the significance of the observed deviation.

The details will become clear from the actual working of examples.

### Example 11

#### Comparison of length of ear-head in Pusa 4 wheat in two successive years

Year	No. of plants	Mean length	Standard deviation	Standard error of mean
1931-32 . . . . .	400	7.88 cm.	1.09	$1.09 \div \sqrt{400} = 0.0545$
1932-33 . . . . .	400	7.82 cm.	0.90	$0.90 \div \sqrt{400} = 0.0450$

Difference in mean length of ear-heads = 0.06 cms.

Standard error of difference =  $\pm \sqrt{(0.0545)^2 + (0.0450)^2}$   
=  $\pm 0.07068$

Therefore  $\frac{\text{difference}}{\text{standard error of difference}} = \frac{0.06}{0.07068}$   
= 0.8489

The difference, being smaller than the standard error of the difference, is not statistically significant; the difference in the mean length of ear-heads in the two samples is such as would occur due to chance.

*Example 12**Comparison of mean heights in two varieties of oats grown under identical conditions*

Variety	No. of plants	Mean height	Standard deviation	Standard error of mean
Scotch Potato oats . . . .	921	104.76 cm.	8.82	$8.82 \div \sqrt{921} = 0.2906$
B. S. 2 oats . . . . .	970	150.21 cm.	5.17	$5.17 \div \sqrt{970} = 0.1659$

Difference in mean heights = 45.45 cm.

Standard error of difference =  $\pm \sqrt{(0.2906)^2 + (0.1659)^2}$   
 =  $\pm 0.3346$

Therefore  $\frac{\text{difference}}{\text{standard error of difference}} = \frac{45.45}{0.3346} = 135.9$ .

The difference being many times the standard error of the difference, we conclude that B. S. 2 oats are really taller than Scotch Potato oats. It is necessary to explain the size of the ratio between the mean difference and its standard error which is considered to indicate significance. We have seen that in the normal curve (page 25) a deviation from the mean of  $\pm 2\sigma$  includes approximately 95 per cent of the items in the sample, in other words, only 5 times in a hundred cases will items differing from the mean by  $\pm 2\sigma$  or more be realized. This may be expressed by saying that the probability of a deviate exceeding  $\pm 2\sigma$  is 5 per cent or  $P = 0.05$ . Considering example 9, we see that the ordinates erected at  $\pm 2\sigma$  cut off from the curve two tails the sum of whose area is 5 per cent of the whole area of the curve, this makes clear the reason for adopting a measure of significance of twice the standard error or as it is called the 0.05 level of significance. This criterion is generally adopted by statisticians and differences between means of less than  $2\sigma$  are considered to be such as would occur 5 times in a hundred from the operation of the chance errors of the experiment. In this book, particularly in the section on yield trials, we generally adopt a stricter criterion of  $P = 0.01$ , which means that only once in a hundred repetitions of the experiment would chance errors of random sampling give a deviation as large as or larger than that observed.

*Bessel's method—*

The determination of the significance of the difference between two means by the use of the formula

$$E_d = \sqrt{E_1^2 + E_2^2}$$

involves the same principle whether the biometrical constant used is the standard error or the probable error. Since, however, the standard error is  $\frac{\sigma}{\sqrt{n}}$  and the

probable error is  $0.6745 \frac{\sigma}{\sqrt{n}}$ , it follows, therefore, that the relationship

$$2 S. E. = 3 P. E. \dots \dots \dots (20)$$

approximately holds good and a deviation of twice the standard error has, therefore, the same significance as three times the probable error. The method of determining significance by the probable errors and the theory of least squares is known as Bessel's method and the criterion of significance generally adopted is that the ratio of the mean difference to the probable error of the difference should be more than 3.2 (see page 30). The method is reliable when the sample is large and when there is no correlation between the different observations as in many chemical and physical determinations, but it is less reliable in its application to field trials where there may be a high correlation between the yielding powers of adjacent plots. We shall revert to this later in our consideration of yield trials.

#### SIGNIFICANCE IN SMALL SAMPLES

In the cases which we have considered up to now it has always been possible to secure a sample of such a size that, given an adequate method of sampling, the sample should be truly representative of the universe. While it is possible to handle a sample of many hundred measurements of a quantitative character, there are other classes of work in which the conditions of the experiment compel us to be satisfied with relatively few observations. It is obvious, for instance, that in a field experiment the number of plots which can be handled is limited and therefore for this type of experiment we have to develop a statistical theory which will allow of satisfactory comparisons being based on small samples. We have seen that in the case of large samples the means of a number of samples from an universe are distributed

normally with a standard deviation of  $\frac{\sigma}{\sqrt{n}}$ ; if  $M_d$  is the deviation of a sample

mean from the mean of the universe, it follows that

$$\frac{M_d}{\frac{\sigma}{\sqrt{n}}}$$

is also distributed normally. In practice, we do not know  $\sigma$  of the universe and we have to substitute for it  $s$ , the standard deviation of the sample, and it is on account of this that our theory has to be modified in the case of small samples. When  $n$  is large, the quantity

$$\frac{M_d}{\frac{s}{\sqrt{n}}}$$

approximates in its distribution sufficiently nearly to the quantity

$$\frac{M_d}{\frac{\sigma}{\sqrt{n}}}$$

to allow of a criterion of twice the standard error being taken as a measure of the 5 per cent level of significance. But when  $n$  is small, the quantity

$$\frac{\frac{M_d}{s}}{\sqrt{n}}$$

is not sufficiently normal in its distribution to allow of the use of this criterion.

*Student's "z".* An anonymous investigator, 'Student'\* has worked out the distribution of a quantity

$$\frac{M_d}{s}$$

and has constructed a table giving for different values of  $n$  the probability integrals of this quantity  $\frac{M_d}{s}$ . This quantity is called Student's  $z$  and is generally expressed as

$$z = \frac{M_d}{s} = \frac{M_d}{\sqrt{\frac{\sum d^2}{n}}} \dots\dots\dots (21)$$

where  $M_d$  = ~~mean difference~~, 'Sample mean' - 'population mean'

$s$  = standard deviation of the ~~difference of the two samples~~, Sample

$\sum d^2$  = sum of squares of the deviations from the mean,

$n$  = size of the sample.

Student's table of  $z$  is available in its original form (*Biometrika* Vol. VI, page 19, and in Pearson's Tables Vol. I, Table XXV, page 36) and as modified by Love (*Jour. Amer. Soc. Agron.* Vol. XVI, page 68). From these tables we can read for any values of  $z$  and  $n$  the probable significance of the observed difference.

#### DEGREES OF FREEDOM

With small samples the best estimate of variability is found by dividing the total sum of squares of deviations not by the number of observations ( $n$ ) but by the *degrees of freedom*, that is, one less than the number of observations ( $n-1$ ). The formula, therefore, for the standard deviation becomes in the case of small samples :—

$$s = \sqrt{\frac{\sum d^2}{n-1}} \dots\dots\dots (22)$$

A simple example will illustrate the difference which this modification makes.

*Example 13.* In Table XV, the yields of Pusa barley type 21 as obtained from 50 small plots are arranged in groups of 5 plots.

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\* Whose work has formed the basis of the study of statistics of small samples.



TABLE XV

*Yield of Pusa barley type 21 in 50 small plots arranged in groups of 5 plots each*

Group	I	II	III	IV	V	Mean
1 . . .	380	618	497	751	396	528.4
2 . . .	352	323	333	188	385	316.2
3 . . .	366	344	544	307	416	395.4
4 . . .	633	630	231	361	280	427.0
5 . . .	575	487	298	510	507	475.4
6 . . .	274	546	464	623	636	508.6
7 . . .	438	295	268	291	388	336.0
8 . . .	418	372	270	293	238	318.2
9 . . .	345	344	340	372	435	367.2
10 . . .	478	377	585	465	605	502.0

The general mean of all the 50 plots is 417.44 grms. and with  $n = 50$ , the value of the standard deviation,

$$s = \sqrt{\frac{824174.32}{50}} = 128.39.$$

If, however, the appropriate degrees of freedom are used as the divisor instead of the actual number of observations, we get

$$s = \sqrt{\frac{824174.32}{49}} = 129.69.$$

It is evident, therefore, that with a sample of 50 observations, in this case, the difference between the values of the standard deviations determined by the use of  $n$  or  $n-1$  is not very large.

Considering now the means of all the 10 groups, of 5 plots each, into which the yields of 50 plots of barley were arbitrarily divided and taking the sum of squares of deviations from the sample mean in each group, we obtain

$$\Sigma d^2 = 60963.8240,$$

as the sum of squares of deviations of all the 10 groups. Since each sample has 5 observations, the total degrees of freedom are  $10(5-1) = 40$  and the calculation

of the standard deviation using 10 samples of 5 plots each becomes

$$s = \sqrt{\frac{60963.8240}{40}} = 123.45,$$

which gives a fairly close agreement to the value 128.39 calculated on the basis of the number of observations ( $n$ ). If when dealing with the squares of deviation from the sample mean we had taken as the divisor  $n = 50$  and not  $n = 10(5-1) = 40$ , we should have got

$$s = \sqrt{\frac{60963.8240}{50}} = 110.42,$$

a value which departs widely from 128.39, the standard deviation of the single large sample. Obviously in the case of the small samples of 5 each the use of degrees of freedom has given us a value of  $s$  closer to the real value.

*Fisher's 't'.* A more recent development of statistical theory for dealing with significance in small samples has been made by Fisher, who has constructed a table of the probability integral of a quantity which he calls ' $t$ '. This quantity differs from Student's ' $z$ ' in that the mean difference is considered in relation to the standard error and not to the standard deviation. Moreover, the standard deviation is calculated not from the size of the sample ( $n$ ) but from the size of the sample less 1, *i. e.*,  $(n-1)$ —this number is called the degrees of freedom. The formula for Fisher's ' $t$ ' is

$$t = \frac{\frac{M_d}{s}}{\sqrt{n}} = \frac{\frac{M_d}{\sqrt{\frac{\sum d^2}{n-1}}}}{\sqrt{n}} = M_d \sqrt{\frac{n(n-1)}{\sum d^2}} \quad (23)$$

In using Fisher's table of ' $t$ ' we enter the table with the degrees of freedom  $(n-1)$  and not with the number of observations ( $n$ ) as in the case of Student's table of ' $z$ '. The ' $t$ ' table gives for degrees of freedom up to 30 and  $\infty$  the probability that an observed value of ' $t$ ' will occur as the result of chance errors. If that probability is low, that is  $P = 0.01$  to  $0.05$ , we conclude that the observed difference,  $d$ , is statistically significant. If, however, the probability observed from the table is higher than  $0.05$ , we conclude that the chance errors of the experiment would be liable to give a difference of the order of magnitude of that observed. Thus, when the degrees of freedom are 7 and the observed value of ' $t$ ' is 3.25, we see from the table that the probability  $P$  lies between 0.01 and 0.02; that is to say, only once or twice in a hundred trials would a value of ' $t$ ' of this size result by chance. On the other hand, with 7 degrees of freedom and ' $t$ ' = 0.6 the value of  $P$  lies between 0.50 and 0.60 denoting that 50 or 60 times in a hundred trial such a value of ' $t$ ' would occur by chance alone. Obviously, therefore, with these degrees of freedom a value of ' $t$ ' of 3.25 or more shows that the observed difference is significant and a value of ' $t$ ' of about 0.6 indicates that the observed difference is not significant.

The importance of the size of the sample in relation to the reliability of the results based on a sample is brought out well by the curve (Fig. 9) which shows the decrease in the value of 't' as the number of degrees of freedom increases.

The value of 't' decreases very rapidly at the  $P=0.01$  level up to about 5 degrees of freedom; with more than 6 degrees of freedom the decrease in the value of 't' is very small and we should, therefore, distrust the reliability of results based on less than samples of five observations.

The value of the standard deviation ~~of the difference~~ calculated on the basis of the number of observations ( $n$ ) can be readily converted into that calculated on a basis of degrees of freedom by multiplying by the fraction  $\sqrt{\frac{n}{n-1}}$ .

In comparing results by Fisher's 't', we must distinguish between cases in which it is legitimate to pair observations and take differences and cases in which observations cannot be paired. When the two sets of observations to be compared relate to samples under identical conditions, except for the variable under study, then the procedure outlined above and illustrated below in Example 14 can be followed. Thus, in the case of yield trials, when we are comparing two treatments in contiguous plots in the same field the yields may be taken in pairs, since the experiment is so designed that the factors affecting the plots are equal in their effect on ~~all the~~ <sup>Pairs of</sup> plots, and the differences between the treatments will not be disturbed thereby. In such a case the degrees of freedom are one less than the number of pairs of observations, that is one less than the number of differences.

It may be, however, that the data are derived from two samples which are independent and which are not related to one another in the sense of being under uniform conditions and in which, therefore, the treatments cannot be paired. In this case the best estimate of the standard deviation which we can make from two samples is given by

$$s = \sqrt{\frac{\sum a_1^2 + \sum a_2^2}{(N_1-1) + (N_2-1)}} \quad \cdot \quad \cdot \quad \cdot \quad (24)$$

but  ~~$t = \frac{d}{s}$~~ ,  
 ~~$\sqrt{n}$~~

therefore, in this case

$$t = \frac{m_1 - m_2}{s \sqrt{\frac{1}{N_1} + \frac{1}{N_2}}} \quad \cdot \quad \cdot \quad \cdot \quad (25)$$

where  $m_1$  and  $m_2$  are the two means and  $N_1$  and  $N_2$  the sizes of the two samples and  $s$  has been calculated by the above formula. In this case the degrees of freedom are

$$(N_1-1) + (N_2-1)$$

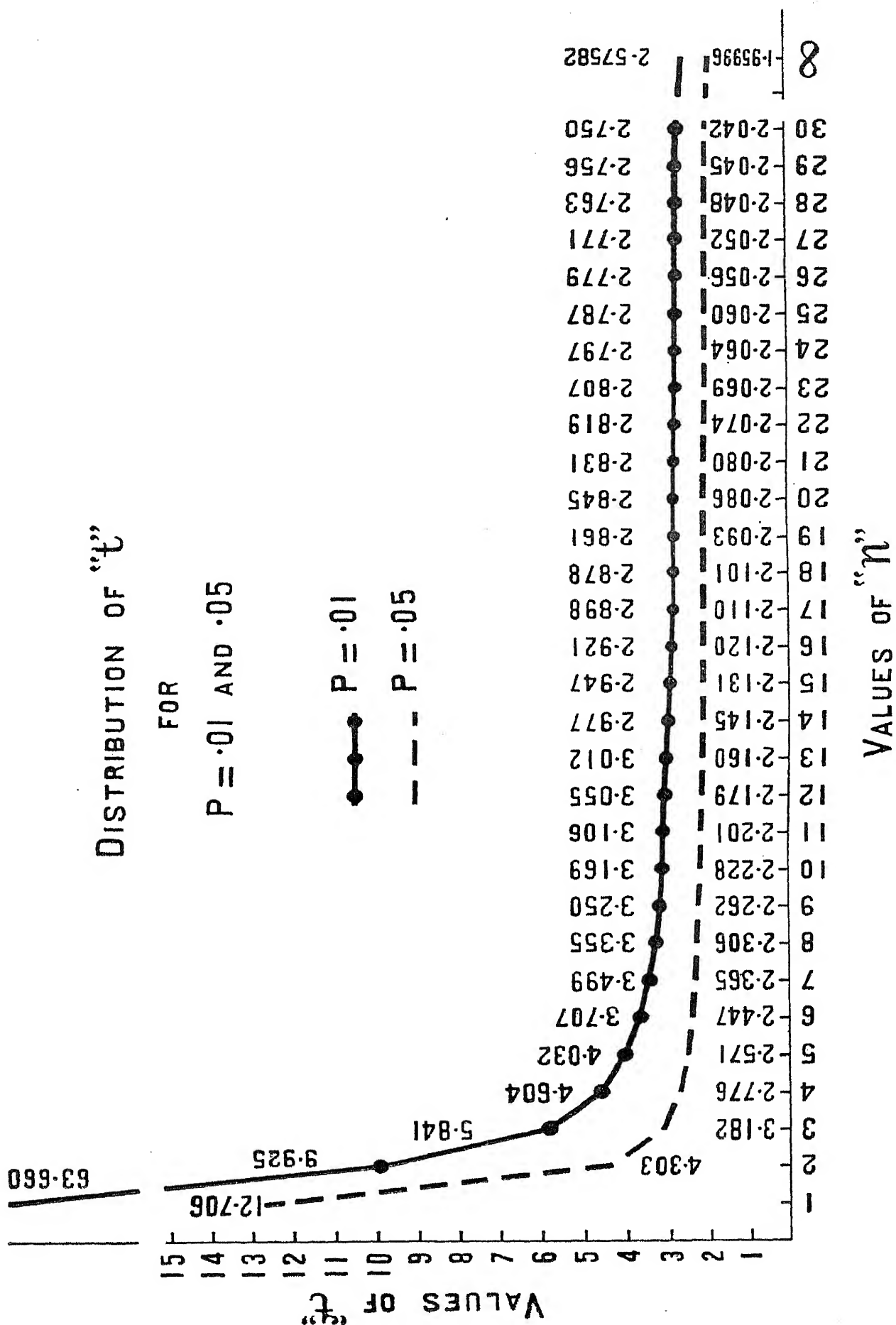


Fig 9.—Showing the distributions of 't' relative to the size of the sample at the P = 0.01 and P = 0.05 levels of significance.

and we enter the 't' table accordingly, that is to say we enter the 't' table with the sum of the degrees of freedom for the two samples.

Mahalanobis has published a modification of the 't' table which he calls the 'f' table and which may be used for the comparison of unrelated samples. The use of this table is explained at page 62.

#### COMPARISON OF RELATED SAMPLES

A few examples will make clear the use of Student's 'z' and Fisher's 't' in determining the significance of mean differences.

*Example 14.* Comparison of yields of two varieties of gram with 6 replications.

TABLE XVI  
*Yields of gram types 17 and 51*

Replications	Yield of type 17 A	Yield of type 51 B	Differences <i>d</i> (B—A)	<i>d</i> <sup>2</sup> .
1 . . .	20.50	24.86	+4.36	19.009
2 . . .	24.60	26.39	+1.79	3.204
3 . . .	23.06	28.19	+5.13	26.317
4 . . .	29.98	30.75	+0.77	0.593
5 . . .	30.37	29.98	—0.39	0.152
6 . . .	23.83	22.04	—1.79	3.204
TOTAL .	152.34	162.21	+9.87	52.479
Means . .	25.39	27.035	+1.645	..

*Student's 'z' test—*

$$\text{Mean difference} = \frac{9.87}{6} = 1.645 \text{ lb.}$$

We now proceed to calculate the standard deviation using the number of observations ( $n = 6$ ) as the divisor and applying the method described at page 22. In the present example since the frequency of each observation is unity,  $f = 1$  and the formula for standard deviation is

$$s = \sqrt{\frac{\sum d^2}{n} - \left(\frac{\sum d}{n}\right)^2} \quad . \quad . \quad . \quad . \quad . \quad . \quad (26)$$

cf. formulæ (2) and (6),

We obtain, therefore,

$$s = \sqrt{\frac{52.479}{6} - \left(\frac{9.87}{6}\right)^2} = 2.458$$

$$z = \frac{\text{Mean difference}}{s} = \frac{1.645}{2.458} = 0.669$$

From Pearson's Table XXV, we see that

when  $n = 6$  and  $z = 0.6$   $P = 0.88129$  and

when  $n = 6$  and  $z = 0.7$   $P = 0.91085$

In the present case, therefore, since  $z = 0.67$ , the probability  $P$  will lie between 0.88 and 0.91. Taking  $P$  as equal to 0.90 we see that the odds are 90 : 10 or 9 : 1 against the difference as great as <sup>or greater than</sup> the observed difference occurring due to chance alone. Such odds, however, are not sufficiently high to indicate a real difference in yielding power between the two varieties. Love has published tables which give directly the odds that an observed difference is significant. From Love's Table we see that in the present example

when  $n = 6$  and  $z = 0.65$  the odds are 8.62 : 1 and

when  $n = 6$  and  $z = 0.70$  the odds are 10.2 : 1,

a result which agrees substantially with the above calculation.

*Fisher's 't' test—*

In applying this test we must remember that the standard deviation of the difference must be calculated on the basis of the degrees of freedom. As already explained this could be done by multiplying the value of the standard deviation obtained for Student's 'z' by the fraction  $\sqrt{\frac{n}{n-1}}$ . In this case, therefore,

$$s = 2.458 \times \sqrt{\frac{6}{6-1}} = 2.6925$$

$$t = \frac{\frac{d}{s}}{\sqrt{\frac{1}{n}}} = \frac{\frac{1.645}{2.6925}}{\sqrt{\frac{1}{6}}} = 1.4962$$

Entering Fisher's 't' table with 5 degrees of freedom, we find that our observed value of 't', viz. 1.496, is very close to the value 1.476 which lies at the  $P = 0.2$  level of significance, this means that approximately 20 times in a hundred repetitions of the experiment would a value of 't' as large as <sup>or larger</sup> the observed value result from chance alone, and the odds therefore are only 4 : 1 against the observed difference being due to chance alone. These odds are approximately half those given by the application of Student's 'z' method. This is because in calculating probability Student's 'z' table considers only one tail of the curve ( $P = 0.90$  and one tail =  $1 - 0.90 = 0.10$ ). Actually a difference of the observed magnitude might be

either positive or negative and therefore the probability of occurrence of a value of 'z' equal to 0.67 is given by  $2(1-0.90) = 0.20$ , which agrees with  $P = 0.2$  given by the 't' method. Fisher's table of 't', therefore, considers positive and negative differences and takes account of both tails of the curve [Tippet, 1931].

*Critical difference.*—Since Fisher's 't' is

$$t = \frac{\frac{d}{s}}{\sqrt{n}}$$

then for any value of 't' corresponding to a definite level of probability we have a significant or critical difference

$$d = t \times S. E. \quad (27)$$

In the present example the standard error (S. E.) is

$$S. E. = \frac{2.6925}{\sqrt{6}} = 1.099.$$

With 5 degrees of freedom at the 1 per cent level of probability,  $t = 4.032$ . Therefore at the  $P = 0.01$  level of significance the critical difference ' $d$ '  $= 4.032 \times 1.099 = 4.4312$  but our observed difference is only 1.645 and this being less than the critical difference is not significant at the 1 per cent level. Similarly at the  $P = 0.05$  level, we find that the critical difference is 2.826 and therefore the observed difference, 1.645, is not significant even at the 5 per cent level.

It is convenient, particularly in experiments such as yield trials, to consider one variable as the control and to express the observed differences as a percentage of the mean of the control. The critical difference may then also be expressed as a percentage of the mean of the control and the significance of the observed difference estimated on a percentage basis. In this experiment, type 17 is taken as control and its mean yield is 25.39 lb. The observed mean difference between the two types in the experiment is 1.645 lb., therefore—

$$\text{the percentage difference} = \frac{1.645 \times 100}{25.39} = 6.48 \text{ per cent}$$

$$\text{and the percentage critical difference at } P = 0.01 \text{ level} = \frac{\overset{4.4312}{\cancel{4.4329}} \times 100}{25.39} = 17.46 \text{ per cent.}$$

The observed percentage difference, being less than the percentage critical difference, is not statistically significant at the  $P = 0.01$  level.

*Example 15.* Improvement in milk yields by special treatment of cows.

Experiments in the agricultural section at Pusa have been carried out to determine the effect of special handling and feeding on milk yields in the Sahiwal herd. A group of cows were subjected to special treatment and the milk yields under this

treatment was compared with previous best lactations of the same cows. The following data are taken from the results :—

TABLE XVII

*Average daily milk yield in lbs. per cow*

Identity No. of the cow	Average milk yield per day in lbs.		Difference $d$	$d^2$
	Under special treatment	For previous best lactation under normal treatment		
555 . . .	16.3	16.1	0.2	..
562 . . .	24.4	16.2	8.2	
564 . . .	22.0	6.1	15.9	
566 . . .	29.2	16.6	12.6	
575 . . .	25.6	8.8	16.8	
577 . . .	19.5	6.8	12.7	
586 . . .	18.1	9.9	8.2	
589 . . .	20.2	10.6	9.6	
591 . . .	25.1	9.8	15.3	
597 . . .	26.8	12.2	14.6	
TOTAL .	..	..	114.1	£ 1529.03

*Student's 'z' test.*—Mean difference =  $114.1 \div 10 = 11.41$  lbs.

$$s = \sqrt{1529.03 \div 10 - (11.41)^2} = 4.766$$

$$\therefore z = \frac{11.41}{4.766} = 2.394.$$

From Pearson's Table XXV, we find that the appropriate value of the probability for this value of  $z$  is 0.99997. Therefore, the odds are 99997 : 3 against a difference of the magnitude of the observed difference being due to chance. In other words, we infer that the increased milk yield is produced by the special treatments of the cows.

*Fisher's 't' test.*—Mean difference =  $114.1 \div 10 = 11.41$  lbs.

$$s = s. \sqrt{\frac{n}{n-1}} = 4.766 \times 1.0535 = 5.0210$$



$$S. E. = \frac{5.0210}{\sqrt{10}} = 1.588$$

$$\therefore t = \frac{d}{S. E.} = \frac{11.41}{1.588} = 7.1761.$$

Expected value of 't' for  $P = 0.01$  and  $n = 9$  is 3.250. Therefore, the observed value of 't', viz., 7.1761 which is much greater than the expected value of 3.250 indicates very high significance in favour of the yields obtained by the special treatments of the cows.

Expressing the observed mean difference and the critical difference as percentages of the mean of the control, i.e., the mean of the previous best lactation, we obtain --

$$\text{the percentage difference} = \frac{11.41 \times 100}{11.31} = 100.09 \text{ per cent.}$$

$$\text{and the percentage critical difference at the } P = 0.01 \text{ level} \\ = \frac{(3.250 \times 1.588) 100}{11.31} = 45.63 \text{ per cent.}$$

An increase of 100.09 per cent which is greater than the percentage critical difference thus shows a significant difference in milk yields by special treatment of the cows.

This experiment is, in its statistical aspect, strictly comparable with the classical experiment of Cushney and Peebles on the soporific effect of the optical isomers of hyoscyamine hydrobromide, which is used as an example in Student's paper [*Biometrika*, VI, page 19] and by Fisher in his book (page 105). In both the experiments two treatments were applied to the same group of patients.

#### COMPARISON OF INDEPENDENT SAMPLES

Let us suppose that the above figures (Table XVII) had been obtained by subjecting two different groups of cows to the two treatments, one group to the normal and one to the special treatment; the experiment would have been less well controlled because it is probable that individual responses to the treatments would to a certain extent be correlated. Thus, in the first instance, when only one group of animals is used for the two treatments, we should expect that a high-yielding individual under one treatment would be a high-yielding individual under the other treatment, whereas, using two groups of animals, individual variations in the yielding power of animals in each group, apart from the effects of the treatments, will have a powerful influence.

If the data are derived from two groups which are not strictly comparable, that is, if the two groups of observations are independent of each other, then the standard deviation is calculated by dividing the sums of squares from the two samples by the total number of degrees of freedom contributed by them; 't' is then calculated by dividing the difference of the two means by the standard error and entering the

table of 't' with degrees of freedom equal to the sum of the degrees of freedom from the two samples. Taking the above figures to represent two different groups of *San* cows, we have—

TABLE XVIII

*Comparison of milk yields in Sahiwal cows*

Treatment	Size of sample	Mean yield in lb. $m$	Sum of squares $\sum d^2$	Variance $\frac{\sum d^2}{N-1}$	(Std. error) <sup>2</sup> $\frac{\sum d^2}{N(N-1)}$
1. Special treatment.	10	22.72	155.216	17.2462	1.7246
2. Normal treatment.	10	11.31	134.189	14.9100	1.4910

Let  $m_1$  and  $m_2$  be the two mean values based on samples of size  $N_1$  and  $N_2$  and let  $S_1^2$  and  $S_2^2$  be the two corresponding variances. Then 't' is given by the equation—

$$t = \frac{m_1 - m_2}{\sqrt{\frac{(s_1)^2}{N_1} + \frac{(s_2)^2}{N_2}}}$$

$$\text{Standard error} = \frac{s}{\sqrt{N}}$$

$$(S.E.)^2 = \frac{s^2}{N}$$

Then 't' should be calculated by formula (24) & 't' determined by formula (25) which reduces in this case to

$$t = \frac{m_1 - m_2}{\sqrt{\frac{\sum d_1^2}{N(N-1)} + \frac{\sum d_2^2}{N(N-1)}}}$$

or if  $s$  has been calculated by formula (24) we can apply formula (25) to determine 't'.

Substituting the required values, we have,

$$t = \frac{22.72 - 11.31}{\sqrt{1.7246 + 1.4910}} = 6.364.$$

Entering the table with  $n = 18$ , the sum of the degrees of freedom of the two samples, we find that the observed value of 't' lies beyond the 0.01 level of significance and hence the difference is statistically significant.

In examples such as this when  $N_1 = N_2 = N$ , we can substitute for the above formula for determining the value of 't' the formula given below:—

$$t = \frac{(m_1 - m_2) \times \sqrt{N}}{\sqrt{S_1^2 + S_2^2}} \quad (29)$$

In the present case, therefore—

$$t = \frac{(22.72 - 11.31) \times \sqrt{10}}{\sqrt{(17.2462 + 14.91) \times 32.1562}} = \frac{11.41 \times \sqrt{10}}{5.671} = 6.364.$$

The student should note that with independent samples the value of the standard deviation of the difference ( $Sd$ ) obtained from the formula  $Sd = \sqrt{(S_1)^2 + (S_2)^2}$  is 5.671 and is very different from the value 5.02 obtained by direct calculation. If we apply formula (29) we have from Table XIV,

$$\Sigma d_1^2 = 155.216 \text{ and } \Sigma d_2^2 = 134.189$$

$$\text{therefore } s_d = \sqrt{\frac{155.216 + 134.189}{(10 - 1) + (10 - 1)}} = \sqrt{16.0780} = 4.01,$$

$$\text{hence } t = \frac{(22.72 - 11.31)}{4.01 \times \sqrt{\frac{1}{10} + \frac{1}{10}}} = 6.364.$$

Mahalanobis has published a table which is a modification of Fisher's 't' table and which may be used to determine the significance of differences between independent samples. This table gives the distribution of a quantity ' $f$ ' at various levels of significance—

$$f = \frac{m_1 - m_2}{Sav.} \quad (30)$$

where  $m_1$  and  $m_2$  are the two mean values and  $Sav.$  is the average standard deviation of the samples determined by the formula  $Sav. = \sqrt{\frac{(S_1)^2 + (S_2)^2}{2}}$

In the case of milk yields, we have

$$Sav. = \sqrt{\frac{17.246 + 14.910}{2}} = \sqrt{16.0780} = 4.01,$$

a value which agrees with that obtained by applying formula (29). It follows, therefore, that

$$f = \frac{11.41}{4.01} = 2.8454.$$

From the table of  $f$ , which is entered with  $n$  equal to size of sample, the number of differences in this case, we find that when  $n = 10$ , the value of  $f = 1.287$  at the  $P = 0.01$  level. The observed value of ' $f$ ' is, therefore, significant at the low level of one per cent and hence we conclude that the difference in milk yields is significant.

From the ' $f$ ' table we can read directly the size of the samples required for a difference of given significance. Thus, in our example with  $f = 2.8454$ , we require

The relationship between " $f$ " and " $t$ " is given by—

$$\begin{aligned} f &= \frac{m_1 - m_2}{Sav.} \\ \text{and } Sav. &= \sqrt{\frac{(S_1)^2 + (S_2)^2}{2}}, \\ \text{whereas, } t &= \frac{m_1 - m_2}{\sqrt{S_1^2 + S_2^2}} \sqrt{n} \\ &= \frac{m_1 - m_2}{Sav.} \sqrt{\frac{n}{2}} \\ &= f \sqrt{\frac{n}{2}} \\ \text{and } \therefore f &= t \sqrt{\frac{2}{n}}. \end{aligned} \quad (31)$$

$$f = 6.364 \sqrt{\frac{2}{10}} = 2.8454.$$

The method of Mahalanobis ("f" table) appears to be more accurate since it is based on Fisher's "t" criterion.

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## CHAPTER VII

## CORRELATION

In our statistical study of variation, we have, upto the present, considered only the measurement of variation in a single variable. It often happens, however, that changes in one variable are accompanied by changes in another and that a definite relation exists between the magnitudes of the changes in each variable. In other words, there is an association or correlation between the two variables. Thus in our example of variation in the length of ear-head and number of grains per head in wheat we find that heads of greater length possess a higher number of grains.

When two variables change together in such a way that a fixed increase in one variable is accompanied by a fixed increase in the other, the variables are said to be positively correlated. A perfect example of correlation is the relationship between the changes in temperature and the length of an iron bar. For every single degree rise in temperature, the length of the bar increases by a fixed amount and the temperature and the length of the bar are positively correlated. In biological measurements, the relationship between two variables is not likely to be so clear cut and definite as this, but it is obvious that certain characters may be expected to show very strong correlation. For instance, in general we should expect a strong positive correlation between the heights of human beings and their weights and this has, of course, been found to exist. Again, if stature is a heritable character in human beings, we should expect a positive correlation between the heights of parents and their children and this has also been demonstrated. Should an increase in one variable go hand in hand with a decrease in the other ~~then~~ these two variables are said to be negatively correlated. If there is no mutual relationship between two variables then they are said to be independent or uncorrelated.

## THE CORRELATION TABLE

The correlation table expresses the relationship between the two variables. For example, the number of grains and the length of heads in Pusa 12 wheat are entered up in a table (Table XIX) in such a way that one set of variable is arranged vertically and the other horizontally. In the class of 15 grains to the ear, for instance, there are 2 ears with an average length of 5.5 cm. ; 1 with 6 cm. ; 8 with 6.5 cm. ; 2 with 7 cm. ; 3 with 7.5 cm. ; and 1 with 8 cm. This shows how in a total of 17 ears with an average number of 15 grains to the ear the variation in length is distributed. In this way all the 400 ear-heads are grouped together according to their respective lengths and the number of grains per ear. The shape of the distribution indicates the extent and the nature of the correlation ; the more elliptical the distribution the stronger the correlation. If the long axis of the ellipse slopes from left to right the correlation is positive ; negative correlation is

indicated when the long axis of the ellipse slopes from right to left, and if the distribution in the correlation table is not markedly elliptical the quantities are not correlated (Fig. 10).

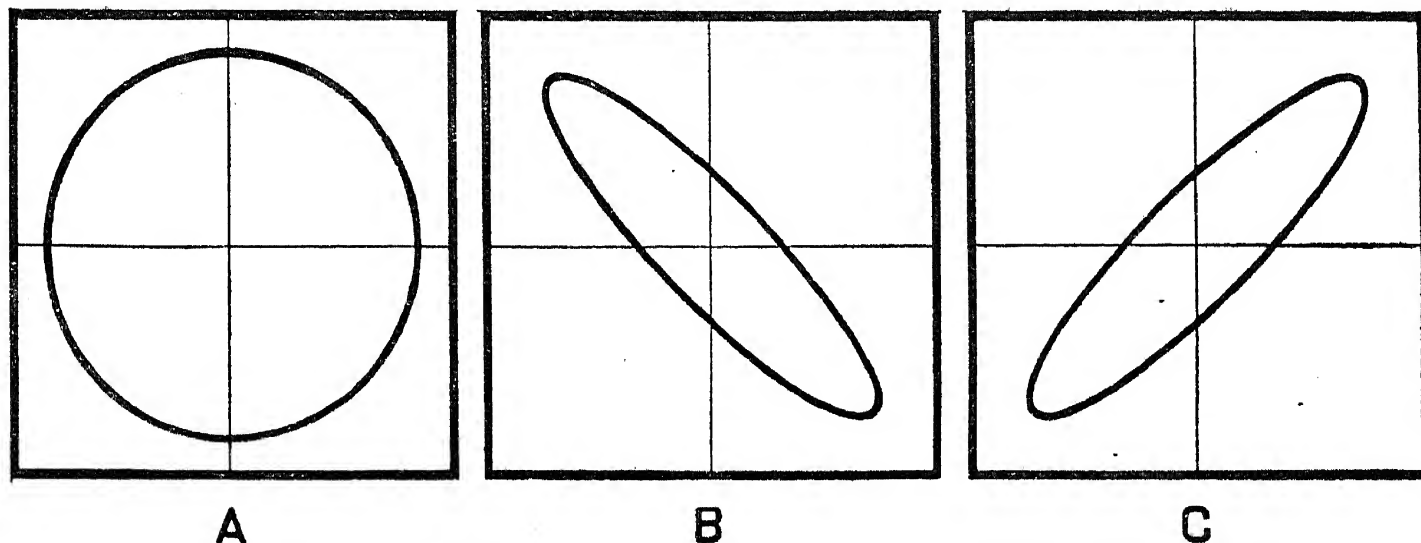


Fig. 10.—Dispersion of data in correlation table. (A) uncorrelated, (B) positive, and (C) negative

Table XIX shows the distribution of the data in the correlation of length of head and the number of grains per head in Pusa 12 wheat, and from the nature of the ellipse enclosing the area covered with the data it is obvious that there is strong positive correlation.

#### COEFFICIENT OF CORRELATION

The intensity of correlation is measured by a coefficient, usually indicated by the symbol  $r$ , which is computed according to the formula:—

$$r_{xy} = \frac{\sum (f. \delta_x \cdot \delta_y)}{N \cdot \sigma_x \cdot \sigma_y} \quad (32)$$

where  $r_{xy}$  is the coefficient of correlation of the variables  $x$  and  $y$ ;  $\delta_x$  is the deviation of the  $x$  variables from the mean of  $x$ ;  $\delta_y$  is the corresponding deviation in the  $y$  variables from the mean of  $y$ ; and  $\sigma_x$  and  $\sigma_y$  are the standard deviations of  $x$  and  $y$  respectively. The item  $\frac{\sum (f. \delta_x \cdot \delta_y)}{N}$  is called the mean product moment.

The direct application of this formula is very laborious but a good short-cut method is described below, and can be used with reliance.

In this short-cut method the lowest class value in each variable is taken as the 'arbitrary origin' and higher class values are considered in serial order as deviations from this. Thus for the length of ear-heads the smallest class value is 5.5 cm. and taking this as the arbitrary origin equal to 0, the class value of 6 cm. is a deviate of 1, the class value of 6.5 is a deviate of 2, the class value of 8.5 cm. is a deviate of 6, and the largest class value of 13.5 is a deviate of 16. A similar process

TABLE XIX

*Coefficient of correlation between the length of head and the number of grains per ear in Pusa 12 wheat, 1930-31 (short-cut method)*

No. of grains. y	Dev. from A. O.	Length in cm. X————→												f. y.	Σ(fdx. dy)					
		5.5	6.0	6.5	7.0	7.5	8.0	8.5	9.0	9.5	10.0	10.5	11.0			11.5	12.0	12.5	13.0	13.5
10	→	1																	1	0
15	↘ 0	(0) 1																	17	40
20	1	(0) 2	(1) 1	(2) 8	(3) 2	(4) 3	(5) 1												25	264
25	2		(6) 4	(8) 4	(10) 6	(12) 5	(14) 4	(16) 2	(24) 22	(27) 13	(30) 4	(33) 2							86	1929
30	3			(12) 1	(15) 3	(18) 18	(20) 1	(24) 3	(28) 12	(32) 27	(36) 38	(40) 22	(44) 15	(48) 5	(52) 1		(60) 1		125	4552
35	4							(30) 6	(35) 3	(40) 6	(45) 9	(50) 18	(55) 12	(60) 13	(65) 8	(70) 2			77	3980
40	5									(48) 1	(54) 5	(60) 10	(66) 7	(72) 10	(78) 13	(84) 3	(90) 3	(96) 3	55	3924
45	6											(70) 1	(77) 1	(84) 3	(91) 1	(98) 2	(105) 1		9	791
50	7																(120) 1	(128) 2	4	480
55	8																	(144) 1	1	144
9	9																			
fx		3	1	8	6	8	11	32	42	58	65	55	37	31	24	7	6	6	400	16054



is carried out for the class values for the number of grains. In the correlation table, to each square containing a number expressing the frequency of that particular variate, we add a number which is the product of the deviations from the two arbitrary origins. For example, in the class containing ear-heads of a class value of 10 cm. and number of grains of 30 the deviations from the arbitrary origins are 9 and 4 respectively and their product, 36, is shown in brackets and is called the 'INDEX NUMBER'. The sum of the products of the frequencies and the index numbers are shown in the last column to the right of the table,  $(\sum f. d_x.d_y)$ . The total of this column divided by the size,  $N$ , of the sample is called the mean product moment from the arbitrary origin. A correction has to be applied to this number because deviations have been taken from arbitrary origins and not from the true means and because the actual class intervals have not been used in the calculations; this correction is the product of the two corrections which were used for the calculations of the means (pages 19 and 20) from the same arbitrary origins. The corrected mean product moment divided by the two standard deviations gives the COEFFICIENT OF CORRELATION. In the present example the calculation is as follows:—

$$\text{Mean product moment from arbitrary origin} = \frac{\sum f. \delta_x.\delta_y}{N} = \frac{16054}{400} = 40.135$$

$$\text{Product of the two correction factors} = C_x.C_y = 8.955 \times 4.11 = 36.805.$$

$$\begin{aligned} \text{Therefore actual mean product moment, } & \frac{\sum (f. \delta_x.\delta_y)}{N} \\ &= \left\{ \frac{\sum f. \delta_x.\delta_y}{N} - (C_x.C_y) \right\} (ix.iy) \\ &= (40.135 - 36.805) (5 \times 0.5) = 8.325. \end{aligned}$$

( $ix$  and  $iy$  being the two class intervals)

Product of the two standard deviations, *i.e.*,  $\sigma_x = 1.4408$  (page 20) and  $\sigma_y = 6.9992$  (page 19.) =  $\sigma_x.\sigma_y = 1.4408 \times 6.9992 = 10.084$ ,

$$\text{therefore } r_{xy} = \frac{\sum (f. \delta_x.\delta_y)}{N.\sigma_x.\sigma_y} = \frac{8.325}{10.084} = 0.8256.$$

The probable error of the coefficient of correlation is expressed by the formula:—

$$E_r = \frac{\pm 0.6745 (1-r^2)}{\sqrt{n}} \quad . \quad . \quad . \quad . \quad (33)$$

The expression  $(1-r^2)$  has been calculated (see Pearson's Table VIII) for all values of  $r$  from  $r = 0.001$  to  $0.999$  and the term  $\frac{0.6745}{\sqrt{n}}$  can be obtained from Pearson's Table V as already explained on page 27. The calculation of the



probable error of the coefficient of correlation, therefore, is a matter of great simplicity. In the present example from Pearson's Tables we find:

$$\frac{0.6745}{\sqrt{400}} = 0.03372,$$

$$\text{and } 1 - (0.8256)^2 = 0.3184;$$

$$\text{therefore } E_r = 0.01074.$$

The standard error of the coefficient of correlation is,  $\frac{(1-r^2)}{\sqrt{n}}$ .

Values of  $r$  always lie between 0 and  $\pm 1$  and the following interpretations are generally given to different values:—

+ 0.5 to + 1.0 indicates high positive correlation

— 0.5 to — 1.0 indicates high negative correlation

+ 0.3 to + 0.4 indicates moderate positive correlation

— 0.3 to — 0.4 indicates moderate negative correlation

smaller than  $\pm 0.3$  indicates low positive or negative correlation.

If only small numbers of individuals are available for the measurement of each character the data are not grouped in classes or even in a table. The following example shows the calculation of the coefficient of correlation between the breakage of rice grains in milling and the temperature of unhusked rice [Rhind and U. Tin, 1933]. In this problem the respective values of  $X$  or temperature, and  $Y$  or breakage percentage, are arranged in parallel columns. The means of  $X$  and  $Y$  are calculated directly from the totals of the columns, and the respective standard deviations are calculated from the sums of squares of each separate entry

$$\text{thus } \sigma_x = \sqrt{\frac{\sum (X^2)}{n} - (\bar{X})^2} \quad (34)$$

where  $\bar{X}$  indicates the mean of  $X$ .

The product moment is obtained by summing the products of  $X$  and  $Y$  and dividing by  $n$  and subtracting from this the product of the two means,  $\bar{X} \cdot \bar{Y}$ . Thus,

$$\text{Product moment} = \frac{\sum (XY)}{n} - (\bar{X} \cdot \bar{Y}) \quad (35)$$

and therefore,

$$r_{xy} = \frac{\frac{\sum (XY)}{n} - (\bar{X} \cdot \bar{Y})}{\sqrt{\frac{\sum (X^2)}{n} - (\bar{X})^2} \cdot \sqrt{\frac{\sum (Y^2)}{n} - (\bar{Y})^2}} \quad (36)$$

Table XX gives the data of  $X$  and  $Y$  and the details of the calculations of  $\bar{X}$ ,  $\bar{Y}$ ,  $\sum (X^2)$ ,  $\sum (Y^2)$  and  $\sum (XY)$ .

TABLE XX

*Correlation of the temperature of unhusked rice (Londi temperature) and the percentage breakage of rice grains in milling*

	Londi Temperature X	Percentage breakage Y	X <sup>2</sup>	Y <sup>2</sup>	XY
1	33.9	27.3	1149.21	745.29	925.47
2	34.6	29.5	1197.16	870.25	1020.70
3	34.5	26.8	1190.25	718.24	924.60
4	36.9	29.5	1361.61	870.25	1088.55
5	37.1	30.5	1376.41	930.25	1131.55
6	37.3	29.7	1391.29	882.09	1107.81
7	28.8	25.6	829.44	655.36	737.28
8	29.6	25.4	876.16	645.16	751.84
9	30.7	24.6	942.49	605.16	755.22
10	31.2	23.6	973.44	556.96	736.32
11	31.6	26.1	998.56	681.21	824.76
12	32.2	24.9	1036.84	620.01	801.78
13	33.4	27.0	1115.56	729.00	901.80
14	33.6	25.6	1128.96	655.36	860.16
15	33.6	26.4	1128.96	696.96	887.04
16	33.9	27.2	1149.21	739.84	922.08
	532.9	429.7	17845.55	11601.39	14376.96

$$\text{Mean of } X = \frac{532.9}{16} = 33.30625$$

$$\text{Mean of } Y = \frac{429.7}{16} = 26.85625$$

Substituting these values in the formula—

$$r_{xy} = \frac{\frac{14376.96}{16} - (33.30625 \times 26.85625)}{\sqrt{\frac{17845.55}{16} - (33.30625)^2} \times \sqrt{\frac{11601.39}{16} - (26.85625)^2}}$$

$$= 0.8482.$$

$$Er_{xy} = \frac{\pm 0.6745 (1-r^2)}{\sqrt{n}} = \frac{\pm 0.6745 \{1-(0.84835)^2\}}{\sqrt{16}} = \pm 0.0473$$

Therefore  $r_{xy} = 0.8482 \pm 0.0473$ .

This shows that there is strong positive correlation between the temperature of unhusked rice and the breakage of rice grains in milling.

### REGRESSION

The coefficient of correlation expresses the degree in which two variables are interrelated, but it is useful to have some method of computing the average expected values of one variable corresponding to particular values of the other, in other words we may wish to calculate the regression of one quantity on the other. The greater the correlation between two sets of variables, the more accurately may the value of one variable be predicted from a known value of the other. In our example of Pusa 12 wheat it is possible to <sup>estimate, for instance,</sup> compute the average number of grains for a particular length of ear-head. This is done by solving an equation the details of the calculation of which are shown below. In the case of Pusa 12 wheat this equation ultimately yields an expression.

$$X = 4.788 + 0.16995 Y \quad Y = -9.4763 + 4.0106 X$$

where  $X$  is the length of ear-head and  $Y$  is the number of grains per ear-head. By substituting the extreme values of  $Y$ , we can obtain corresponding values of  $X$ ; and similarly values of  $Y$  can be calculated for the extreme values of  $X$  and the results plotted in the form of two straight lines which will intersect at a point corresponding to the means of the two variables.

The angular difference between the two lines of regression (Fig. 11) is inversely proportional to the strength of the correlation between the two quantities. If there is no correlation at all the two lines are perpendicular to one another and the mean value of one variable is the same for all values of the other. If the coefficient of correlation is unity there is perfect association and the two lines will coincide.

The details of the calculation of the regression of length of ear ( $X$ ) on the number of grains per ear ( $Y$ ) in our example of Pusa 12 wheat are given below:—

Regression of length of ear ( $X$ ) on number of grains per ear ( $Y$ ) in Pusa 12 wheat (1930-31) is—

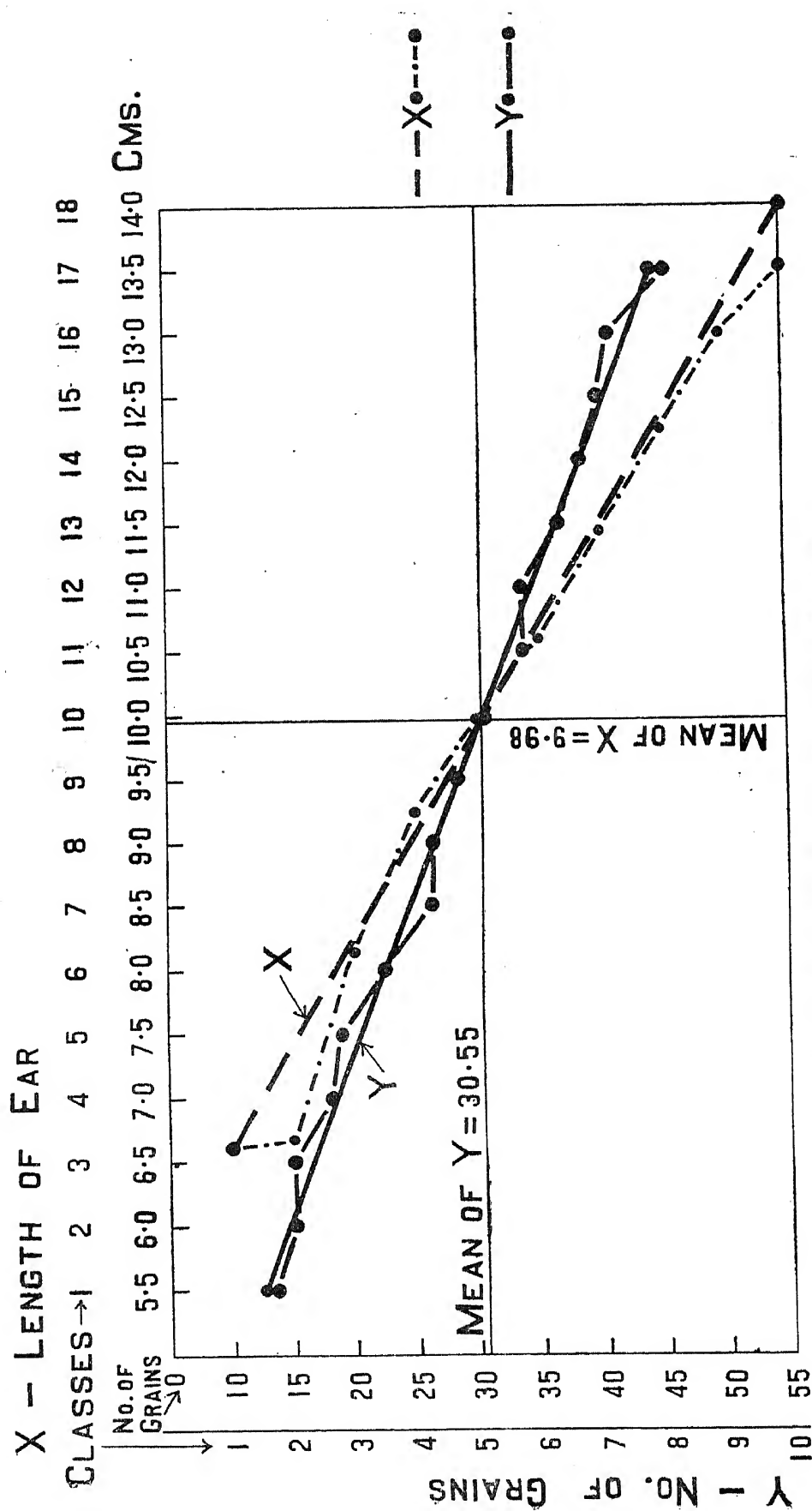
$$\begin{aligned} X &= \left\{ \bar{X} - r_{xy} \cdot \frac{\sigma_x}{\sigma_y} \cdot \bar{Y} \right\} + \left\{ r_{xy} \cdot \frac{\sigma_x}{\sigma_y} \cdot Y \right\} \\ &= 9.98 - 0.8256 \times \frac{1.4408}{6.9992} \times 30.55 + 0.8256 \times \frac{1.4408}{6.9992} \times Y \\ &= 4.7880 + 0.16995 Y. \end{aligned}$$

Substituting the values of the end classes of  $Y$ , i.e., 10 and 55 grains respectively, (Fig. 11) we get—

$$X = 4.7880 + 0.16995 \times 10 = 6.4875$$

$$X = 4.7880 + 0.16995 \times 55 = 14.13525$$

which are the end points of the  $X$  axis.



Regression of number of grains per ear ( $Y$ ) on the length of ear ( $X$ ) is—

$$Y = \left\{ \bar{Y} - r_{xy} \cdot \frac{\sigma y}{\sigma x} \bar{X} \right\} + \left\{ r_{xy} \cdot \frac{\sigma y}{\sigma x} X \right\}$$

$$= 30.55 - \frac{0.8256 \times 6.9992}{1.4408} \times 9.98 + \frac{0.8256 \times 6.9992}{1.4408} \times X$$

$$= -9.4763 + 4.0106 X.$$

Substituting the values of end classes or 5.5 and 13.5 cm. respectively we get—

$$Y = -9.4763 + 4.0106 \times 5.5 = 12.5820$$

$$Y = -9.4763 + 4.0106 \times 13.5 = 44.6668$$

which are the end points of the  $Y$  axis in Fig. 11.

Thus the average value of  $X$  for any value of  $Y$  and *vice versa* can be calculated.

Having obtained the desired straight-line regressions of  $X$  on  $Y$  and  $Y$  on  $X$  as shown in Fig. 11, it may be desired also to obtain the average deviations per class of the various  $x$  and  $y$  arrays as shown by the broken lines plotted in the Fig. 11. The easiest way of doing this is to tabulate the correlation surface as shown in Table XXI and to calculate the mean of each horizontal and each vertical frequency distribution as shown under  $\Sigma yX/fy$ . It will be observed that instead of computing the  $\Sigma yX$  and  $\Sigma xY$  values from the actual class centres for the number of grains and length of ear-heads respectively, these have been obtained from arbitrary class centres of 1, 2, 3, 4, 5.....etc., in order to save arithmetical labour. The values thus obtained are plotted and show the deviation of each class from the straight-line regressions.

The regression coefficients are given by the formulae :—

$$r \frac{\sigma y}{\sigma x} \text{ and } r \frac{\sigma x}{\sigma y}$$

and the equations giving the most probable value of  $y$  for any given value of  $x$  and *vice versa* are obtained by

$$(a) \dots (y - \bar{y}) = r \frac{\sigma y}{\sigma x} (x - \bar{x})$$

$$(b) \dots (x - \bar{x}) = r \frac{\sigma x}{\sigma y} (y - \bar{y}).$$

From equations (a) and (b) it is evident that the expressions,  $r \frac{\sigma y}{\sigma x}$  and  $r \frac{\sigma x}{\sigma y}$  give the tangents of the angles that the lines (a) and (b) make with the  $X$  axis.

Now  $r \frac{\sigma y}{\sigma x}$  represents the rate of increase <sup>in</sup> of  $y$  for unit increase of  $x$ . In our

Pusa 12 wheat example,  $r \frac{\sigma y}{\sigma x} = 4.011$ . This indicates that for every change of one unit of  $x$ , which is the independent variable, there is a corresponding change of 4.011

TABLE XXI

*Correlation surface and average deviation per class in Pusa 12 wheat (1930-31)*

No. of grains.	Length of ear $\times \longrightarrow$																	iv.	$\sum_y X$	$\sum_y X/f.y.$
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17			
1	1																	1	1	0
2		1	8	2	3	1												17	57	3.35
3				4	4	6	5	4	2									25	157	6.28
4					1	3	18	23	22	13	4	2						86	729	8.48
5						1	3	12	27	38	22	15	5	1		1		125	1263	10.10
6							6	3	6	9	18	12	13	8	2			77	863	11.21
7									1	5	10	7	10	13	3	3	3	55	709	12.90
8											1	1	3	1	2	1	1	9	122	13.69
9														1		1	2	4	64	16.00
10																	1	1	17	17.00
$\sum x.$	3	1	8	6	8	11	32	42	58	65	55	37	31	24	7	6	6			
$\sum y$	5	2	16	16	22	37	138	182	272	331	312	212	197	161	49	43	49			
$\sum y/f.y.$	1.7	2.0	2.0	2.66	2.8	3.5	4.31	4.33	4.69	5.1	5.67	5.73	6.35	6.7	7	7.2	8.1			

in  $Y$ , the dependent variable. For each increase of 0.5 cm. in the length of ear-heads in Pusa 12 wheat the number of grains increases on an average by 2.0053

Similarly  $r \frac{\sigma x}{\sigma y} = 0.16995$  gives the rate of increase ~~of~~ <sup>in</sup>  $x$  for unit increase of  $y$ .

In the example in question the number of grains per ear-head, which is the independent variable, increases by 5 and the dependent variable increases at an average rate of 0.84975 cm.

### SIGNIFICANCE OF AN OBSERVED CORRELATION

The probable error of the coefficient of correlation is given by the formula : —

$$P. E._r = \frac{\pm 0.6745 (1-r^2)}{\sqrt{n}}$$

This tells us that it is an even chance that any subsequent determination of the coefficient, based on a similar sample, will fall within these limits. The formula given for the probable error of the coefficient of correlation is accurate only when  $n$  is not very small, since with large samples and moderate and small values of  $r$  the correlation is distributed normally about the true value of the coefficient of correlation of the universe. With small samples, less than a hundred, the value of  $r$  is often very different from the true value and the factor  $1-r^2$  is consequently in error. Therefore, tests of significance based on the probable error or standard error for small samples are not always very reliable.

In testing the significance of an observed correlation it is necessary to determine the probability that such a value would occur in a random sample drawn from a population in which the two variables are not correlated. If this probability is small, *i.e.*, at the 5 per cent. or at the 1 per cent. level of significance, the correlation coefficient may be considered to be significant. Significance depends upon the size of  $r$  and upon the number of observations in the sample from which  $r$  is calculated, and can readily be determined by the application of Fisher's 't' table or by a special table (Fisher's Table V-A) which he has elaborated. If  $n'$  be the number of pairs of observations on which the correlation is based, and  $r$  the correlation obtained, then,

$$t = \frac{r}{\sqrt{1-r^2}} \cdot \sqrt{n'-2} \quad . \quad . \quad . \quad (37)$$

and it may be demonstrated that the distribution of 't' so calculated, will agree with that given in the table. The 't' table is entered with degrees of freedom equal to the number of pairs of observations less 2, *i.e.*,  $n'' = n' - 2$ .

Fisher's Table V-A allows this test to be applied directly from the value of  $r$ , for samples upto 100 pairs of observations. Taking the four definite levels of significance, represented by  $P = 0.10, 0.05, 0.02$  and  $0.01$ , the table shows for each value of  $n$ , from 1 to 20, and then by larger intervals to 100, the corresponding significant values of  $r$ .

*Example 16.*—In 16 pairs of observations the coefficient of correlation between the breakage of rice grains in milling and the temperature of unhusked rice was found to be 0.848 (page 69). What is the probability that such a value would be obtained by random sampling from data in which the variables are uncorrelated?

Applying the formula

$$t = \frac{r}{\sqrt{1-r^2}} \cdot \sqrt{n'-2}$$

we have,

$$r = 0.848$$

and from Pearson's Table VIII, we get  $1-r^2 = 0.2807$ ,

$$\text{therefore, } t = \frac{0.848}{\sqrt{0.2807}} \cdot \sqrt{16-2} = 5.9866.$$

The 't' table must now be entered with degrees of freedom equal to  $n'-2 = 14$  and we find that the expected value of 't' at the  $P = 0.01$  level is 2.977; the observed value, 5.9866 being much greater than the expected value, we conclude that a coefficient of this magnitude would not occur once in a hundred trials as the result of chance errors and therefore, it is significant.

Fisher's Table V-A shows that for 16 pairs of observations at the 1 per cent level of significance a value of  $r$  of 0.6226 or more is significant, the table being entered with  $n = n'-2$ , in this case  $n = 16-2=14$ . Our observed value of 0.848 being much higher we can safely conclude that the correlation is statistically significant.

The distribution of different values of  $r$  at the 5 per cent and the 1 per cent levels of significance for degrees of freedom of 1 to 30 and thence by larger intervals to 100 in simple correlations of two variables is shown in Fig. 12. From these curves it can be seen that with 14 degrees of freedom the significant value of  $r$  at the 1 per cent level is 0.623.

Fisher makes use also of another method of determining the significance of  $r$ , which is based on the following transformation :—

$$z = \frac{1}{2} \log_e \frac{1+r}{1-r} \quad . \quad . \quad . \quad (38)$$

and values of  $z$  corresponding to values of  $r$  are tabulated in his Table V-B. The standard error of  $z$  is simply

$$\sigma z = \frac{1}{\sqrt{n'-3}} \quad . \quad . \quad . \quad (39)$$

and is independent of the value of correlation in the sample under consideration.

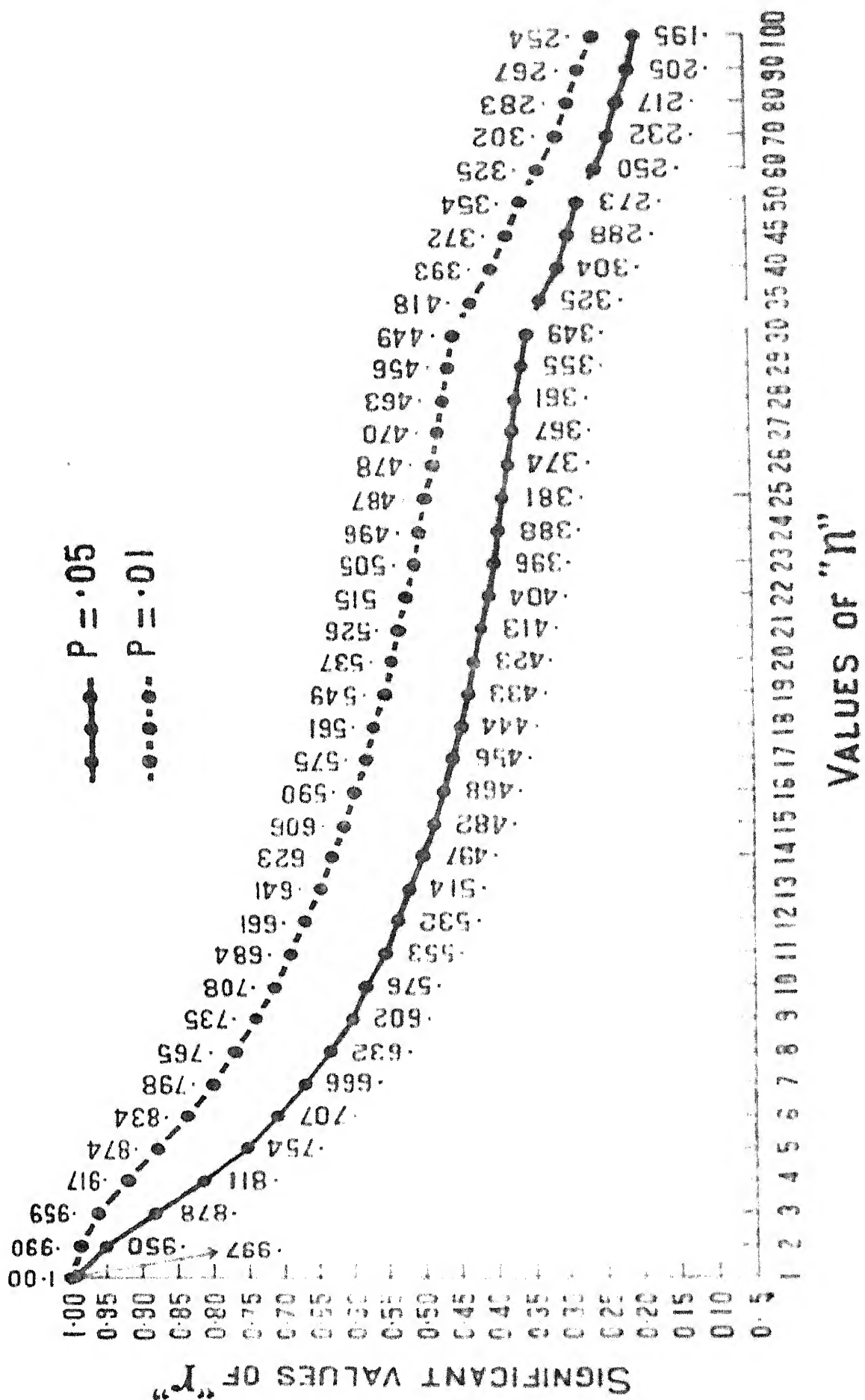
In the case of our example, applying Table V-B, we see that

$$r = 0.8483$$

$$\text{the value of } z = 1.25$$

$$\text{and the standard error of } z = \frac{1}{\sqrt{16-3}}$$





$z$  is a difference between two logarithmic values and to estimate the significance of this difference we must express it in terms of its standard error and refer to the tables of the probability integral. Thus,

$$\frac{z}{S. E._z} = \frac{1.25}{\frac{1}{\sqrt{13}}} = 1.25 \times \sqrt{13} = 4.506.$$

Referring to Pearson's Table II, we find that when this ratio is 4.506 the greater area of the curve cut off by the ordinate is 0.9999966 and the area in the two tails is, therefore,  $2 \times 0.0000034$ . Therefore the observed value of the coefficient of correlation will be exceeded by chance only 68 times in ten million trials.

#### SIGNIFICANCE OF DIFFERENCE BETWEEN TWO OBSERVED CORRELATIONS

Two tests with Taungdeikpan rice in Burma [Rhind and U. Tin, 1933] showed that the coefficients of correlation between the breakage of rice grains in milling and the temperature of unhusked rice were, 0.8912 in a sample of 12 pairs and 0.8482 in a sample of 16 pairs of observations. Are these values significantly different?

This can be determined as follows by transforming the values of  $r$  into  $z$  :—

TABLE XXII

*Correlation coefficients of breakage of rice grains and the temperature of unhusked rice*

Samples	$r$	$z$	$n - 3$	Reciprocal
1st sample . . . . .	0.8912	1.4276	9	0.11111
2nd sample . . . . .	0.8482	1.2496	13	0.07692
Difference . . . . .	0.0430	0.1780	..	Sum 0.18803

We find that the difference in the values of  $z$  is 0.1780 and that the standard error of the difference is, of course, the square root of the sum of the reciprocals of 9 and 13.

Standard error of difference of  $z = \sqrt{0.11111 + 0.07692} = 0.4336$ .

The difference, between the two values of  $z$ , viz., 0.1780, being smaller than the standard error of the difference is not statistically significant.

#### LONG-TIME TRENDS

If it is desired to determine the general inclination of movement in a series, such as average yields per acre or trend of wholesale prices of crops, *e.g.*, rice, wheat, linseed, etc., over a long period of years we may simply represent graphically the upward or downward tendencies as shown in the continuous line chart given in Fig. 13 or if a precise measurement is to be made of the degree or extent of the rise and fall in the course of a series, we may employ special methods, such as have been described by Harper [1930] and other workers.

For series which show a general movement in one direction, the straight line trend of least squares, or the first degree parabola, is usually applied. This straight line is so fitted that the summation of the squares of the deviations from it is less than the summation of the squares of deviations from any other straight line that can be drawn. A concrete example of this is given below and will illustrate the technique involved. If, however, a series shows a marked upward slope and then moves downwards at a decided slope, it cannot properly be represented by a straight line trend but will require the application of the logarithmic, or the second degree parabola. The discussion of the latter method, however, is out of the scope of this book.

*Example 17.*

*Soil fertility experiment in the Pusa Farm.*—413 acres were cropped under a three-year rotation designed to supply grain of cereals, such as oats, maize, etc., and some pulses, as well as the supply of fodder for the upkeep of the pedigree dairy herd. Yields for the 15-year period, 1912-13 to 1926-27, are given in the second column of Table XXIII. The production of grain shows a steady upward tendency during this period. Fit a straight line of least squares to prove that there is a steady upward trend of fertility.

TABLE XXIII

*Straight line trend of the total yield of grain (cereals and pulses) of 13 fields (413 acres) in Pusa Farm \**

Year	Total yields of grain in maunds	Deviation from the point of origin	Deviation squared	Product of deviation and yield	Ordinates of the straight line trend of least squares
	$y$	$x$	$x^2$	$xy$	$Y$
1912-13 . .	3,626	1	1	3,626	3386·2169
1913-14 . .	3,297	2	4	6,594	3583·5383
1914-15 . .	2,987	3	9	8,961	3780·8597
1915-16 . .	4,254	4	16	17,016	3978·1811
1916-17 . .	4,499	5	25	22,495	4175·5025
1917-18 . .	4,662	6	36	27,972	4372·8239
1918-19 . .	4,982	7	49	34,874	4570·1453
1919-20 . .	4,262	8	64	34,096	4767·4667
1920-21 . .	4,381	9	81	39,429	4964·7881
1921-22 . .	6,153	10	100	61,530	5162·1095
1922-23 . .	5,189	11	121	57,079	5359·4309
1923-24 . .	4,536	12	144	54,432	5556·7523
1924-25 . .	7,517	13	169	97,721	5754·0737
1925-26 . .	5,984	14	196	83,776	5951·3951
1926-27 . .	5,183	15	225	77,745	6148·7165
TOTAL .	71,512	120	1,240	627,346	..

\* Data from *Scientific Repts. of the Agr. Res. Inst., Pusa, 1926-27*, p. 73.

The straight line trend may be calculated by solving the two normal equations, (40) and (42).

$$Y = a + b x \dots\dots\dots (40)$$

where  $Y$  is the ordinate of the straight line,  $a$  the starting point,  $b$  the slope of the line and  $x$  the distance from the point of origin. The following normal equations may be constructed for the formula  $Y = a + b x$

$$\Sigma y = a n + b \Sigma x \dots\dots\dots (41)$$

$$\Sigma x y = a \Sigma x + b \Sigma x^2 \dots\dots\dots (42)$$

Substituting the proper values from Table XXIII and solving the two normal equations, we have :—

$$(1) \dots 71512 = 15 a + 120 b$$

$$(2) \dots 627346 = 120 a + 1240 b$$

Eliminating  $a$  by multiplying equation (1) by 8 and equation (2) by 1, we get,

$$(3) \dots 572096 = 120 a + 960 b$$

$$(4) \dots 627346 = 120 a + 1240 b$$

---


$$(5) \dots -55250 = -280 b \text{ [ By subtraction of equation (4) from equation (3). ]}$$

therefore (6)  $\dots 197.3214 = b$ .

In equation (1), we have seen that

$$15 a + 120 b = 71512.$$

Therefore, by substituting the value of  $b$ , we get,

$$15 a + (120 \times 197.3214) = 71512$$

$$\therefore 15 a = 71512 - (120 \times 197.3214)$$

and  $a = 3188.8955$ .

Since  $b = +197.3214$ , we conclude that the slope  $b$  of the straight line trend of least squares is a positive quantity, and hence that the yield of grain from the whole area of 413 acres has shown an upward tendency at an average rate of 197.3214 maunds per year since 1912-13. Now to determine the ordinates of the straight line of least squares we simply have to substitute actual values in the formula  $Y = a + b x$ . For instance the ordinate for 1912-13 is :—

$$\begin{aligned} Y &= a + b x \\ &= 3188.8955 + (197.3214 \times 1) \\ &= 3386.2169 \end{aligned}$$

Similarly the ordinate for 1913-14 is :—

$$\begin{aligned} Y &= a + b x \\ &= 3188.8955 + (197.3214 \times 2) \\ &= 3583.5383. \end{aligned}$$

Similarly the ordinates for the years following are obtained in the same way, the value of  $x$  increasing by 1 for each year above 1912-13, or simply by adding the value of  $b = 197.3215$  to the value of the ordinate of the previous year. These are shown in the last column  $Y$  of Table XXIII.

The actual data in the second column of this table may now be represented graphically as shown by the broken line in Fig. 13; the straight line trend of least squares, plotted on the ordinates shown in column 6, is represented by the thick line.

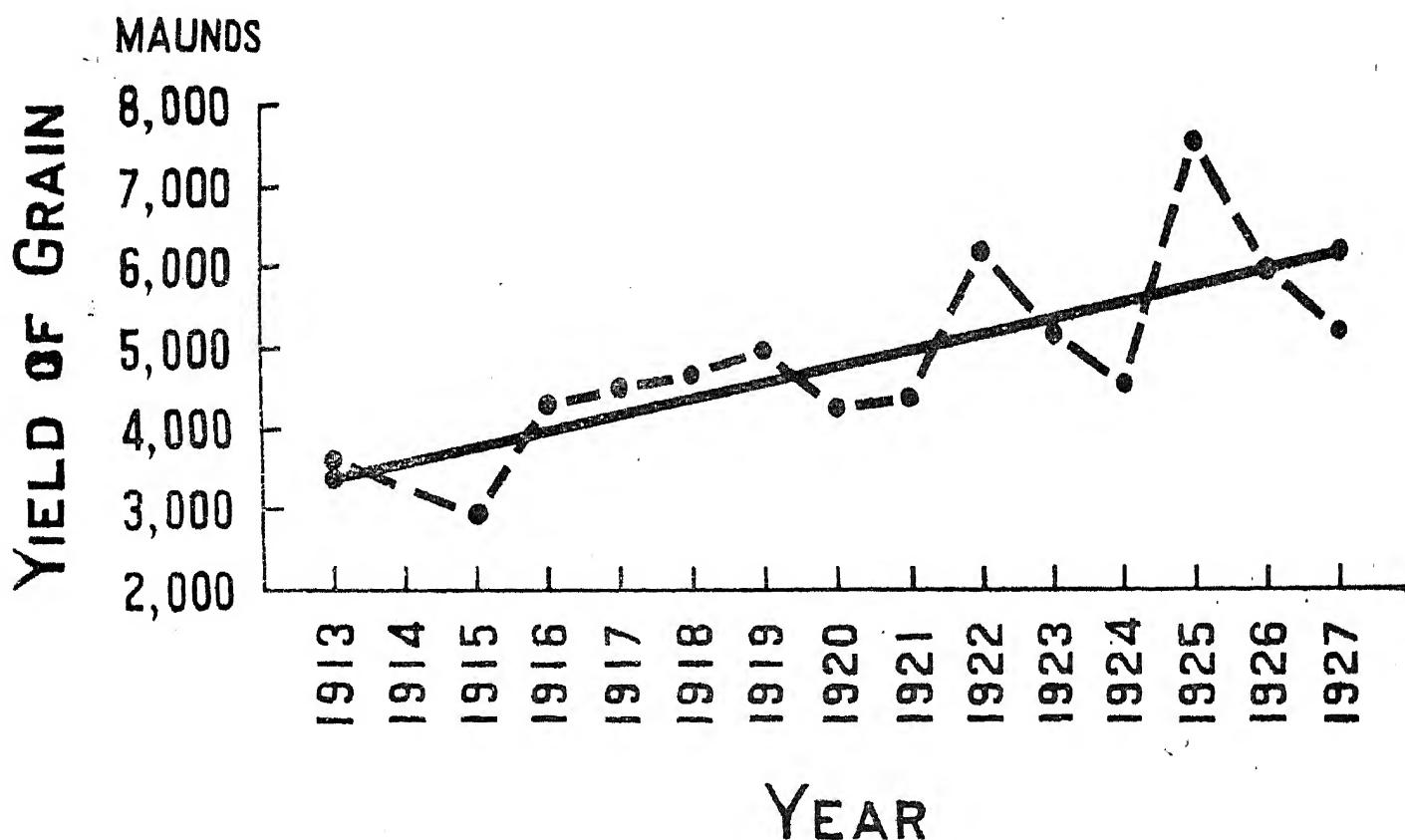


Fig 13.—Straight line trend of the total yield of grain of 13 fields (413 acres) in Pusa Farm, 1912-13 to 1926-27

The trend, therefore, definitely shows a steady upward tendency.

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## CHAPTER VIII

## FIELD TRIALS

Field experiments generally have for their object the testing of the relative yielding powers of different varieties or the comparison of the results obtained by the application of different manures or varying cultural methods. In any case, whether the comparison is between varieties, manures, or cultural methods, the variables under study are referred to as treatments. Thus, in a comparison of yielding power of four varieties of linseed, the experiment contains four different treatments. The testing of varieties in the field presents special difficulties, for in no other type of experiment are there so many factors which are outside the control of the operator. Comparisons of the yields of different varieties grown in the same field in different years are affected by variations in climate. Since particular varieties will respond differently in seasons of heavy and light rainfall and a variety which proves the heaviest yielder in one season may prove inferior under different conditions in the next season. Similarly the effects of different manures may vary with climatic factors. When comparisons are made between yields of different varieties in the same years and in the same field, the varying fertility of different parts of the field introduces an unknown factor into the experiment and it may be said at once that the development of modern methods of conducting field trials has for its object the elimination, as far as possible, of the influence of this unknown variable upon the result of the experiment. The operator can control the manurial treatment, the method of cultivation and the particular varieties sown and in any one year in any particular field all treatments will be subject to the same climatic conditions, but there remains, however, a large and unestimated variation due to differences in the fertility of different parts of the field. This soil heterogeneity may, as is shown in a subsequent chapter, be determined for a particular field and its effects allowed for in subsequent experiments in the same field. Generally, however, the determination of soil heterogeneity in a field involves a lengthy series of experiments and considerations of time and space render it impossible to precede every field trial by determinations of soil heterogeneity.

Since the fertility in a field generally varies in an irregular manner, the distribution of patches of high and low fertility follows no definite plan; in other words, we are generally faced by a random distribution of soil fertility. A common type of soil heterogeneity is one in which there is a distinct drift or gradient from high to low fertility across the field from one side to the other; a fertility gradient may, of course, be combined with irregular variations in fertility. In order to eliminate the effects of the random variation in soil fertility or of the fertility gradient or of both, the experimenter seeks to distribute his treatments about the field in such a way that each treatment shall have an equal chance of experiencing all varying grades of soil fertility. In a broad sense such distribution involves the scattering of treatments about the field at random and the replication of each treatment many

times. The experiment must be designed in such a way as to yield both an efficient comparison of the treatments and an estimation of the statistical significance of the observed differences between treatments. Both these results can be achieved by the replication of treatments in a number of small plots. The design and technique of experiments will vary with the nature of the trial to be carried out but the fundamental principles outlined below will apply to all experiments. It cannot be too strongly emphasized that the success of an experiment depends upon a correct design and care in the field operations. No amount of statistical juggling with the data will compensate for inaccuracy in the field.

### EXPERIMENTAL TECHNIQUE

(1) *Selection of soil.* The field selected for comparative trials should be truly representative of the soil and other conditions under which the crop to be experimented with is normally grown and the land should have been previously cropped in such a manner as to have been kept in an uniform state of productivity. In a plant-breeding station in which fields are generally being used for the study of a large number of cultures of different varieties it is a good practice to rotate a bulk crop with plant-breeding plots in order to keep the land in an uniform condition. The land selected for comparative trials should possess good drainage and should be of average fertility, neither very high nor very low. It should have no large trees in the neighbourhood as they are likely to disturb the growth of the crop by their lengthy root-systems. The previous cropping history of the land should be known; it is obviously unsuitable to take land for varietal trial which has been used in the previous season for a comparison of different manures or *vice versa*.

(2) *Size and shape of plots.* The size and shape of the plot will depend obviously on the nature of the crop to be grown; it is evident that a size and shape which is suitable for a crop such as wheat will not give a reliable result with a crop such as sugarcane. It may be laid down as a general rule that small areas of land are more uniform in fertility than large areas and hence trials with cereal crops (*e.g.*, wheat, barley, etc.) should be carried out with individual plots of not more than one-fortieth of an acre, while for sugarcane, plots of about one-twentieth of an acre are of a suitable size.

Increasing plot size results, in the case of small cereals, in a rapid decrease in the variability of the yield, up to a size of one-fortieth of an acre; with plots larger than this size the continued decrease in the standard deviation is very small as is shown in Fig. 14. If it is desired to increase the area under an experiment it is much more effective to do so by increasing the number of replications which may be easily done with plots of small area. The smaller the area of the unit the larger the number of replications it is possible to include in the experiment and for this reason, with cereal crops it is possible to obtain accuracy with areas of plots much smaller than one-fortieth of an acre.

Plots are always rectangular in shape but may range from squares to long strips. The square shape has the advantage that the edge effect is reduced to a minimum.



but for certain crops (*e.g.*, those in which the individual plant is large, as pigeon-pea and sugarcane) the long plot has practical advantages, such as ease in cultivation, etc.

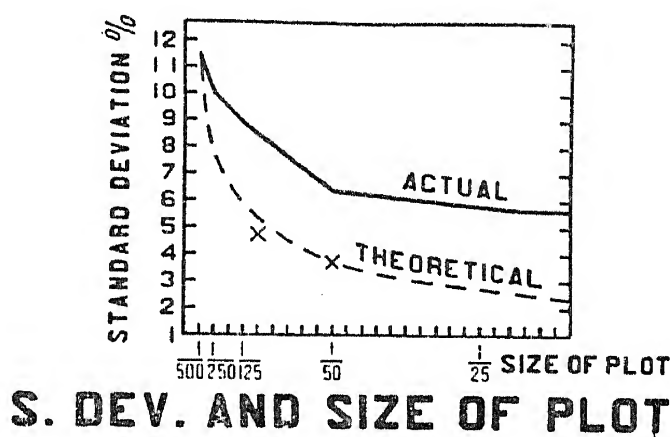


Fig. 14. Reduction in standard deviation due to increase in the size of plots (After Hayes and Garber, p. 74).

(3) *The arrangement of treatments.* The selection of the best arrangement is greatly facilitated if there is any information as to the distribution of fertility in the field. This can be obtained by a previous testing of the field as a whole by growing a bulk crop and harvesting it in small rectangular plots. If the yields of these are plotted on a graph paper in the position in which they appear on the field a fairly accurate idea of the relative merits of different parts of the field can be obtained, and any tendency for the fertility to grade in one or more directions is at once obvious. It is, of course, not always possible to carry out such a preliminary test and the modern systems of distributing treatments in a field are designed to give effective results even when such information is not available.

(a) *Paired plots.* When only two varieties or two manurial or other treatments are to be compared they may be laid out in long strips across the field in the order

*A A B B A A B B A A . . . . .*

and Fisher's "*t*" or Student's "*z*" methods of determining the significance of the results utilized. The pairing of contiguous plots is an essential condition in the use of Student's "*z*" test in field trials. A lay-out, such as this, is a useful method for comparing one treatment with another, since it gives paired contiguous plots which, by reason of their much greater length than breadth, should each of them experience the varying grades of fertility in the field to a corresponding degree, or at least the experience of contiguous plots in this respect should be more or less similar. The object of arranging the paired plots in the series *AB BA AB BA . . . . .* and not in the series *AB AB AB AB . . . . .* is, of course, to insure that in any pair of plots one treatment is not always on the same side of the other and thereby experiencing the advantage of a possible fertility gradient. Beaven's half-drill method is based on the same principle and the statistical handling of such a lay-out is relatively simple.

(b) *The chess-board method.* This method is convenient when several treatments are to be compared and the quantity of seed available is limited as may well



be the case at a relative early stage in the evolution of new types in plant-breeding investigations; it is most suitable for use with small cereals such as wheat and barley. The arrangement of plots in a trial with 6 treatments is

*A B C D E F A*  
*B C D E F A B*  
*C D E F A B C*  
*D E F A B C D*  
*E F A B C D E*  
*F A B C D E F*  
*A B C D E F A*  
 and so on.

The size of plots in the case of small cereals is generally 4 ft.  $\times$  4 ft. and by cutting away a 6-in. border the yield is actually taken from a square yard. In this type of experiment the same number of seeds must be sown in each plot and hence the field work is laborious. The advantage of this method is that with such small plots a large number of replications can be distributed in a field and therefore each treatment should have an equal chance of experiencing the natural advantages or disadvantages of the variations in the fertility of different parts of the field.

(c) *The Latin Square*.—This arrangement resembles the chess-board but there are two restrictions and the arrangement of types is not systematic. The restrictions are that there are as many replicates of each treatment as there are treatments, the plots being arranged in a rectangle with as many rows as columns so that each treatment occurs once in each row and once in each column. This provides for a double elimination of the influence of soil heterogeneity in two directions at right angles to one another, *i.e.*, between columns and rows. In the following two arrangements an incorrect and a correct method of distributing treatments in a  $5 \times 5$  Latin Square is illustrated :—

Incorrect method  
of replication.

*A B C D E*  
*B A C D E*  
*C B A E D*  
*D E B A C*  
*E C D B A*

Correct method  
of replication.

*A B C D E*  
*D C B E A*  
*E A D C B*  
*B D E A C*  
*C E A B D*

Although in the incorrect method there is randomization of treatments in the row, but there is none in the column, since in three of the columns the same treatment occurs in adjacent plots. In the correct method of randomization it will be found, however, that the same type occurs once only in the row and in the column, thereby greatly reducing the influence of soil heterogeneity. The Latin square is only a square in a conventional sense, for the actual shape of the plots

may be made to suit the available area and the crop. Plots may be square or rectangular but precision is lost if they are too long and narrow. The statistical analysis of this lay-out as well as that of randomized blocks dealt with below is generally carried out by Fisher's Analysis of variance.

(d) *Randomized blocks.* When several treatments are to be compared the experiment may be arranged in the form of randomized blocks by dividing the experimental area into regular blocks of equal areas in each of which the treatments are distributed at random, each treatment occurring once only in each block. The individual blocks can be distributed in any manner, that is, either parallel to each other across the field or in two directions. It is an advantage if the area can be divided in two directions at right angles to one another so that all the blocks are not lying side by side. The advantage of the randomized block arrangement is that replication is secured, and at the same time it is easy to distinguish the soil variation within the blocks from that between the different blocks, since the total yield of any block is comparable with that of any other block in that each contains one plot of each treatment. The variations in the total yields of blocks may, therefore, be ascribed to soil differences and these gross differences can be eliminated from the comparisons of the different treatments. The fertility of the field may be broadly classified into—

(1) A major fertility variation usually marked by a fertility gradient ; and

(2) Sporadic fertility variations, not systematic but distributed in patches.

The object of dividing the field into blocks is to calculate the variance in yield due to the effects of the first type of fertility and eliminate this variance for arriving at an estimate of variance due to chance error. The object of randomizing the treatments within the blocks is to eliminate the effects of the sporadic variations in fertility.

The randomized block method allows of any number of replications, unlimited by the number of treatments involved. It has also the advantage that if a part of the experiment is damaged by some agricultural disaster (*e.g.*, insects, floods, water-logging, etc.) it is possible to discard entirely one or two blocks without destroying the entire experiment. A reduction in the number of replications would, of course, lead to a larger standard error in the experiment but would at least furnish a result of some value.

(4) *Replications.* The errors in a field experiment may, broadly speaking, be divided into three classes :—

1. Errors due to soil heterogeneity ;
2. Errors due to faulty technique ; and
3. Errors due to chance.

The errors due to soil heterogeneity we seek to eliminate by the random arrangement of plots on the methods previously outlined and by the replication of treatments many times over. The errors due to faulty technique vary inversely with the skill, care and experience of the experimenter ; with the trained worker they should be non-existent or negligible. The errors due to chance come in unsuspected by the experimenter and are governed by the mathematical laws of probability,

the principles of which have been described in previous chapters. Chance errors can, therefore, be estimated by submitting the data to an appropriate mathematical analysis and in general, as has been shown, the importance of chance errors in an experiment varies inversely as the number of replications, since the formula for the estimation of error contains the square root of the number of observations as the divisor—

$$P. E. M = \pm \frac{0.6745 \sigma}{\sqrt{n}}$$

Thus, to double the <sup>accuracy</sup>~~significance~~ of a result obtained from 25 plots, we should have to take into the experiment 100 plots, since  $\sqrt{25} = 5$  and  $\sqrt{100} = 10$ . The larger the number of replications, however, the greater the area of land and consequently the greater is the variability of productivity. Fig. 9, which shows the relationship between the values of 't' and the size of the sample brings out clearly how the reliability of the result depends upon the number of observations. Not less than five replications are essential for reliability. The size of sample necessary for a given degree of precision can be determined by Mahalanobis' Table of "f".

(5) *Randomization*. Systematic arrangement of plots in any definite order may increase or decrease our estimates of the experimental error. This is more pronounced if the lay-out happens to be such that the long axes of the plots lie parallel to a fertility gradient running from one side of the field to the other. Hence, a representative distribution of the strains in an experimental field brought about by randomization is bound to give a much more reliable result than mere systematic repetition. In other words, the randomization of plots gives an even chance for the random distribution of the treatments to spread themselves over the different fertility patches. Such a random distribution gives statistically a valid basis for estimating the standard error due to chance on which comparisons between treatments depend.

An easy method of randomization has been suggested by Fisher and is best explained by means of an example. Suppose that we have to randomize a set of five treatments in a randomized block experiment with six replications. To do this a set of random two-figure numbers is selected, the chance opening of a book furnishes a ready means of selection. Suppose the first random number from the book is 28; dividing this by 5, the number of treatments, we get a remainder of 3, and we allot treatment number three to the first plot in the first block. Suppose now that the second number chosen at random is 36, this on division by 5 gives a remainder of 1 and treatment number 1 will go into the second plot of the first block. We proceed in this way for all the plots in every block subject to the restriction that the same treatment may not occur more than once in any block. If the number chosen is a multiple of 5, it is considered to indicate treatment number 5.

Tippett [1927] has published 10,000 numbers of random digits and Mahalanobis [1933] has recently described the use of random sampling numbers in agricultural experiments and has published a list of 2,000 random numbers. The student is referred to his paper for the method of using these numbers.

(6) *Number of treatments.* It frequently happens that at the conclusion of an investigation on plant-breeding, the research worker possesses a large number of types of which he requires to investigate the productivity. It is obvious that the ordinary yield trial cannot be carried out with 50 to 100 varieties. Some rough preliminary selection must be made of the types which are of good yielding capacity. The usual practice in the Botanical Section at Pusa is to take the seed weight from 50 plants selected at random from a small plot of a variety. This is a method suitable for large plants such as pigeon-peas. To do this, it is necessary to grow small plots of all the types in the same field, the field being of average fertility. Another method is the *rod-row* test in which three rod-rows of each type are grown. The lateral rows are discarded for border effect and the yields of the central rows only are compared. This method is suitable for small plants, *e.g.*, cereals like wheat and barley. The number of replications which can be given to a rod-row trial depends somewhat on the number of varieties involved.

From the results of such a preliminary trial it should be possible to select a number of the most productive types and these may be tested further in randomized blocks with unit plots of about  $\frac{1}{100}$ th acre, with, if possible, 5 replications. The number of replications which can be given will naturally depend upon the number of types which have been selected for testing, but at this stage this number should not exceed 20. A further selection can now be made of about half a dozen of the best yielding types and a standard yield trial in randomized blocks or latin squares, with unit plots of the necessary area, as previously described, and with replications of certainly not less than 5.

(7) *Duration of yield trial.* It is a fact well known to agriculturists that the yield of a crop from the same field varies in different seasons according as the climate is favourable or not to the particular crop. It will be readily understood that different varieties of a crop will, in addition to their morphological differences, possess physiological differences (*e.g.*, water requirements), which will give an advantage to certain types in certain seasons. It is obviously impossible to carry on varietal trials for such a number of years as to ensure that the varieties experience a random sample of the climatic variations. In India, the most potent variable in the climate is the size and distribution of the monsoon rainfall and successive years may vary very greatly in the availability of soil moisture. Varieties which yield well in years of adequate moisture may fail to show high yields in seasons in which soil moisture is deficient and *vice versâ*. It is, therefore, desirable to carry out standard yield trials for at least three successive seasons in the same locality in order that a deduction may not be based upon the results of a single season which has possibly been unduly favourable to a particular type. It is an advantage also to repeat a yield trial in the same season in different localities under different conditions of soil and climate. In this way by yield trials under different climatic and soil conditions, it is possible to form an estimate of the suitability of particular varieties for particular agricultural areas.

(8) *Sowing and rate of seeding.* Sowing should be done in rows at a spacing appropriate to the particular crop and must be carefully supervised; a definite

number or quantity of seed has to be sown in each sub-plot. Broadcasting should never be done. If from some outside cause, *e.g.*, white ants, plots become damaged, it is permissible to fill in gaps by re-sowing at early stages. Since the size of seed in different varieties may vary considerably, the seed-rate used in sowing a variety trial should not be based upon weight alone but upon the number of seed per unit weight and the percentage germination. An estimation of the number of seeds per gramme or any other unit of weight can easily be obtained and a sowing rate which will give an approximately equal number of seeds per plot calculated. This sowing rate can be increased or decreased according to the percentage germination of individual types. Thus, in three varieties of linseed taken for a yield trial, the number of seeds per gramme and the seed-rate per acre calculated to give approximately the same number of seeds per acre are as shown below :—

Variety	No. of seeds per gm	Seed-rate per acre in ounces
Type 12 . . . . .	222	256
Hybrid 69 . . . . .	187	305
Hybrid 64 . . . . .	156	366

The germination percentage of all three varieties being between 93 and 95 per cent, the calculated seed-rate did not require further adjustment. Wherever possible the same number of plants should be used in each plot in an experiment. This may be achieved either by sowing equal number of seeds or by thinning the seedlings.

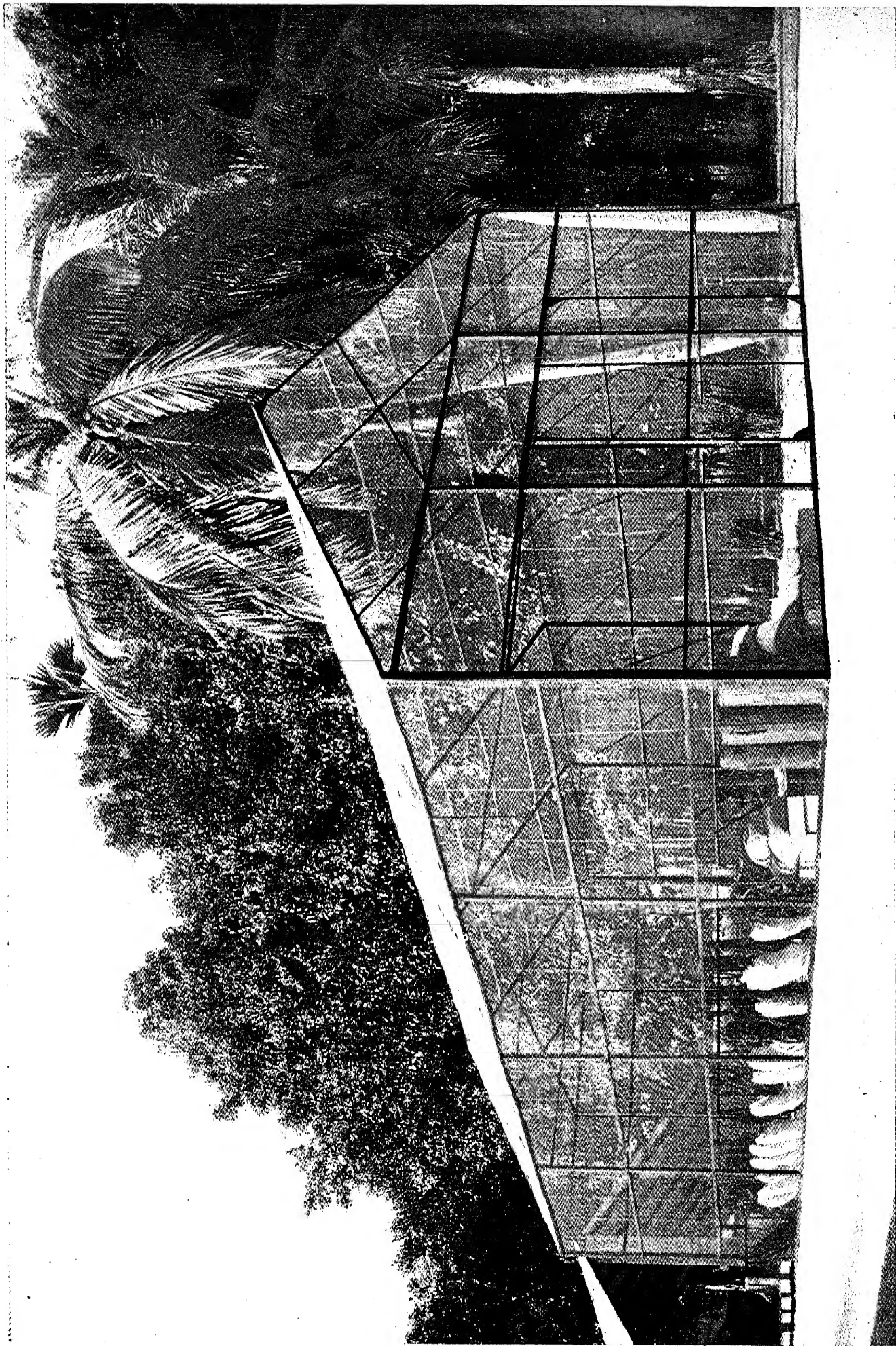
(9) *Harvesting.* The harvesting of yield trials demands skilled labour and strict supervision. The student should remember that it is easy to lay down, at sowing time, yield trials on a scale which it is impossible for the available staff to handle with accuracy at harvest. Subject to this limitation there are certain general precautions which must be observed.

(10) *Border effect.* A strip on each edge of a plot must be cut away and thrown out of the experiment in order to ensure that the yield of each plot is only taken from such an area as is unaffected by the growth of contiguous varieties or by the presence of adjacent grass, drains or unsown land. The central area from which the yield is finally taken is referred to as the ultimate plot.

Each plot should be threshed *in situ*, if possible, by hand. Hence the desirability of not having plots so large as to render this impossible. In the Botanical Section at Pusa the usual practice is to spread a large cotton (drill) sheet on the plot after cutting the crop and to rub by hand or beat out the grain with small wooden sticks on this sheet.

Seed should be thoroughly sun-dried until a uniform weight is reached. In Pusa this is usually done on a threshing floor which is enclosed and covered by a wire-net house. (Fig. 15.)





*Fig. 15.* Photograph of netted threshing floor.



Since the total weights are of a smaller order than is generally the case in farm yields a more accurate and sensitive type of weighing machine than is generally used in agricultural practice is required.

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## CHAPTER IX

## STATISTICAL INTERPRETATION OF FIELD EXPERIMENTS

Since the number of plots which can be handled in a field experiment is relatively small, the statistical interpretation of such experiments is generally a case of the statistics of small samples, a subject which has been dealt with in a previous chapter (page 50). The modern designs for the lay-out of field experiments have already been explained and we have now to consider the available statistical methods which can be used in their interpretation.

The principles underlying the statistical interpretation of field trials will be readily grasped by the student who has studied the chapter on the significance of means. All statistical determinations of significance are fundamentally based upon the table of the probability integral and involve a comparison of the observed differences between means with a function of the variance, either the standard deviation or the standard error or the probable error. The methods that have been used in the past and those which have been more elaborated recently may be summarized as follows :—

(1) *Bessel's method*. This has already been described in some detail and consists in determining the means, standard deviations and the probable errors of the means of the yields of the two varieties under comparison ; and then determining the ratio of the difference of the two means to the probable error of the difference. If this ratio be more than 3.2 times the probable error of the difference the results are said to be statistically significant. This method is reliable where there is no correlation between the different observations as in many chemical and physical determinations, but it is less reliable in its application to field trials where there may be a high correlation between the yielding powers of adjacent plots.

(2) *Student's method*. This method requires the tabulation of differences between the yields of two treatments and the determination of the ratio :—

$$\frac{\text{Mean difference}}{\text{Standard deviation of the difference}} = 'z'$$

From this ratio the odds are read off from tables which express the probability in terms of the value of 'z'. The most important condition of this method is that the lay-out should be such as to lend itself to the pairing of observations, *e.g.*, yields of adjacent plots, and that there must be a number of replications to obtain significant results. The pairing of plots allows for the influence of the correlation between the yielding powers of adjacent areas.

(3) *Fisher's 't'*. This method differs from Student's method in that the standard deviation is determined from the degrees of freedom and a quantity 't', which is the ratio of the mean difference to the standard error of the difference, is used for the estimation of the significance. The table of 't' is entered with the degrees of freedom and gives the probability that the observed value of 't' will occur as the result of chance errors.

(4) *Engledow and Yule's method*. This is a method by which a number of varieties can be compared at the same time. The standard deviation of the difference of two sets of comparable plots from their means is determined and the standard error of this is calculated by the formula

$$S.E._d = \frac{\sigma d}{\sqrt{n}} \quad . \quad . \quad . \quad . \quad . \quad . \quad (43)$$

The significance of the difference between two varieties is given by the ratio of the difference of their means to the standard error. If this ratio be greater than 2.1, the odds against the observed difference being due solely to errors of random sampling are 30 : 1, and the result is considered to be significant. This method was elaborated as an extension of Student's 'z' and resembles the use of Fisher's 't'. The calculation is rather cumbersome and the method has been superseded by Fisher's Analysis of Variance.

(5) *Fisher's Analysis of Variance*. This is the method utilized for interpreting the results obtained from randomized blocks and Latin Square methods of lay-out. According to this the total variation in the yields of plots is sub-divided into different parts representing :—

- (1) the effect of variety or treatment,
- (2) differences in yield between different blocks, or different rows or columns, and
- (3) residual effect representing the random or uncontrolled variation of the experiment.

The residual variance furnishes the basis for the calculation of the experimental error. As the variance due to soil differences (due to blocks or to rows and columns) is eliminated in the analysis of variance, the residual variance, that is that due to chance errors, furnishes a better criterion for estimating significance than the standard deviation calculated without any such elimination. Moreover, the variances are based on those degrees of freedom which contribute to the errors, produced by the various factors causing the variation in yield.

A few actual examples of the different methods of lay-out and the application of the various statistical tests to their results will illustrate the principles described in the previous pages.

## A YIELD TRIAL BY THE METHOD OF PAIRED PLOTS

*Example 18**Purpose of experiment.*—Varietal trial with gram.*Field.*—Botanical Section, No. 5.*Varieties.*—*A* and *B*.*Lay-out.*—Paired strips — *A A B B A A B B*.....*No. of replications.*—6.*Size of plot.*—84' × 13' = 1,092 sq. ft.*Size of ultimate plots after removing the necessary borders.*—80' × 9' = 0.0165 acre.

Plot No	Type	Yield in lb.
1	A	26.90
2	B	34.59
3	B	32.80
4	A	25.62
5	A	25.36
6	B	32.29
7	B	30.49
8	A	26.39
9	A	27.16
10	B	34.34
11	B	35.36
12	A	23.32

Fig. 16.—Plan of a yield trial with paired plots (*Example 18*)

TABLE XXIV

*Plot yields and calculations of Example 18*

Replication	Yield of A	Yield of B	Difference $d$ (B — A)	$d^2$ (B — A) <sup>2</sup>
1	2	3	4	5
1	26.90	34.59	7.69	59.1361
2	25.62	32.80	7.18	51.5524
3	25.36	32.29	6.93	48.0249
4	26.39	30.49	4.10	16.8100
5	27.16	34.34	7.18	51.5524
6	23.32	35.36	12.04	144.9616
TOTALS .	154.75	199.87	45.12	372.0374
Means . . . .	25.79	33.31	7.52	..

In the above table the differences in the yields of paired plots are taken down in column 4 and in this case all of them happen to be positive differences in favour of variety *B*. The sum of these differences divided by the number of pairs of plots gives the mean difference of the experiment. Column 5 shows the square of each of these differences. The sum of all these squares gives the value of  $\sum d^2$  required for determining the standard deviation of the difference.

$$\text{Mean difference} = \frac{45.12}{6} = 7.52 \text{ lb.}$$

$$\begin{aligned} \text{Standard deviation of the difference, } s &= \sqrt{\frac{\sum d^2}{n-1} - \frac{(\sum d)^2}{n(n-1)}} \\ &= \sqrt{\frac{372.0374}{6-1} - \frac{(45.12)^2}{6(6-1)}} = 2.5588 \end{aligned}$$

$$\text{Standard error} = \frac{s}{\sqrt{n}} = \frac{2.5588}{\sqrt{6}} = 1.044$$

$$\text{Fisher's "t"} = \frac{\text{Mean difference}}{\text{Standard error}} = \frac{7.52}{1.044} = 7.23$$

Expected value of "t" from Fisher's table, entering the table with  $n-1 = 5$

For 1 per cent level of significance = 4.032

and for 5 per cent level of significance = 2.571.

The observed value of “ $t$ ”, viz., 7.23 being higher than the expected values we conclude that the difference in the yields of varieties  $B$  and  $A$  is statistically significant and hence  $B$  is significantly a higher yielder than  $A$ .

Since  $t = \frac{d}{S.E.}$  as shown above,

the critical difference  $d = t \times S.E.$ ,

and for 1 per cent level  $d = 4.032 \times 1.044 = 4.2094$ . As the observed difference of 7.52 lb. is greater than the expected critical difference of 4.2094, it is obvious again that the difference is statistically significant.

Type  $A$  was an established variety of proved yielding power and type  $B$  was a new variety which was being tried against  $A$ . It is, therefore, convenient to express the critical difference and the mean difference between  $A$  and  $B$  as percentages of the mean yield of the control,  $A$ .

We obtain in this case critical percentage difference at the 1 per cent level

$$= \frac{\text{critical difference} \times 100}{\text{Mean } A} = \frac{4.209}{25.79} \times 100 = 16.32 \%$$

Similarly mean difference as a percentage of the mean yield of the control  $A$  is

$$\frac{\text{Mean difference} \times 100}{\text{Mean } A} = \frac{7.52}{25.79} \times 100 = 29.16 \%$$

i.e., variety  $B$  has out-yielded variety  $A$  by 29 per cent and this percentage is significant since the critical percentage difference is only 16.

Applying Student's ‘ $z$ ’ test we obtain

$$\text{Standard deviation of the difference, } \sigma d = \sqrt{\frac{372.0374}{6} - \left(\frac{45.12}{6}\right)^2} = 2.3358$$

$$\text{Therefore, Student's 'z' } = \frac{7.52}{2.3358} = 3.219$$

From Love's Tables, we see that the odds against a difference of the magnitude of that observed being due to chance alone are 2499 : 1 when  $z = 3.2$  and  $n = 6$ , indicating that variety  $B$  is significantly superior to variety  $A$  in yielding power.

Student's ‘ $z$ ’ may, as in the present instance, sometimes give very high odds but from a practical standpoint when using Student's ‘ $z$ ’ significance at 30 : 1 or the stricter criterion of 100 : 1 is sufficient.

#### A YIELD TRIAL BY THE CHESS-BOARD METHOD

##### Example 19

*Purpose of experiment.*—Varietal trial with barley.

*Field.*—Botanical Section, plot 1— $A$ .

*Varieties.*—Barley Types 21 ( $A$ ), 20 ( $B$ ), 14 ( $D$ ) and local ( $C$ ).

*Lay-out.*—Chess-board.

No. of replications.—25.

Size of plot.—4' × 4' = 16 sq. ft.

Size of ultimate plots.—3' × 3' = 9 sq. ft.

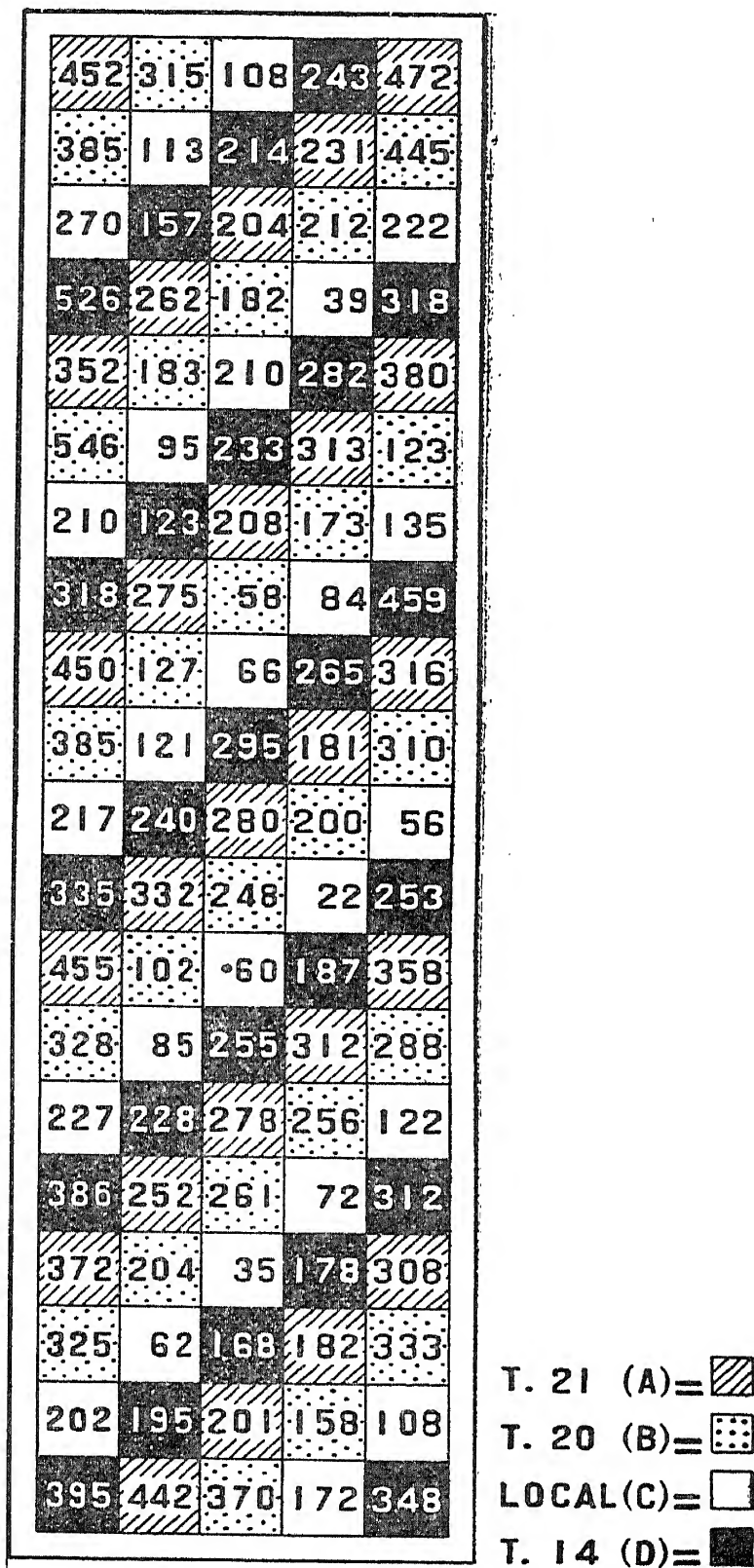


Fig. 17.—Plan of Chess-board lay-out with barley (Figures in each square indicate yields in grms.)

The significance of the differences in yielding power between the four types can be readily determined by Engledow and Yule's method [Shaw and Bose, 1929]. This method allows the comparison of all the types which are included in the experiment. Table XXV gives the yields of all plots, the mean yield of each type and the deviation of each plot yield from the mean of the type. Table XXVI shows the differences ( $d$ ) between these deviations, and the squares ( $d^2$ ) of these differences, for all pairs of contiguous plots in each comparison. Thus the deviation of the yield of plot 1, Type  $A$ , from the mean yield of all plots of Type  $A$  is 137.3, and similarly for plot 2, Type  $B$ , the deviation from the mean yield for all  $B$  plots is 124.3; the difference " $d$ " in this case is, therefore,  $137.3 - 124.3 = 13$  and " $d^2$ " is 169. The value of " $d^2$ " is calculated in this way for all possible pairs of contiguous plots. In this experiment there are six possible comparisons of types.

$A$  with  $B$

$B$  with  $C$

$C$  with  $D$

$A$  with  $C$

$A$  with  $D$

and  $B$  with  $D$

with 25 pairs in each comparison. There are, therefore, 150, *i.e.*,  $25 \times 6$ , values of " $d^2$ " and the standard deviation of the difference is obtained by the formula

$$\sigma d = \sqrt{\frac{\sum d^2}{150}}.$$

The standard error " $S.E_d$ " is given by  $\frac{\sigma d}{\sqrt{n}}$  where  $n$  is the number of plots

under each type, in this case 25.

TABLE XXV

*Calculation of statistical significance by Engledow and Yule's Method in Example 19*

Yields in grms				Deviations from the mean			
A	B	C	D	A	B	C	D
452	385	270	526	137.3	124.3	145.5	249.5
352	546	210	318	37.3	285.3	85.5	41.5
450	385	217	335	135.3	124.3	92.5	58.5
455	328	227	386	140.3	67.3	102.5	109.5
372	325	202	395	57.3	64.3	77.5	118.5
262	315	113	157	-52.7	54.3	-11.5	-119.5
275	183	95	123	-39.7	-77.7	-29.5	-153.5
332	127	121	240	17.3	-133.7	-3.5	-36.5
252	102	85	228	-62.7	-158.7	-39.5	-48.5
442	204	62	195	127.3	-56.7	-62.5	-81.5
204	182	108	214	-110.7	-78.7	-16.5	-62.5
208	58	210	233	-106.7	-202.7	85.5	-43.5
280	248	66	295	-34.7	-12.7	-58.5	18.5
278	261	60	255	-36.7	0.3	-64.5	-21.5
201	370	35	168	-113.7	109.3	-89.5	-108.5
231	212	39	243	-83.7	-48.7	-85.5	-33.5
313	173	84	282	-1.7	-87.7	-40.5	5.5
181	200	22	265	-133.7	-60.7	-102.5	-11.5
312	256	72	187	-2.7	-4.7	-52.5	-89.5
182	158	172	178	-132.7	-102.7	47.5	-98.5
472	445	222	318	157.3	184.3	97.5	41.5
380	123	135	459	65.3	-137.7	10.5	182.5
316	310	56	253	1.3	49.3	-68.5	-23.5
358	288	122	312	43.3	27.3	-2.5	35.5
308	333	108	348	-6.7	72.3	-16.5	71.5
Mean 314.7	260.7	124.5	276.5	..	..	..	..



TABLE XXVI

Example 19.—Engeladow and Yule's Method—Differences of deviations for all possible pairs of treatments

Pairs A and B		Pairs B and C		Pairs C and D		Pairs A and C		Pairs A and D		Pairs B and D	
$d$	$d^2$	$d$	$d^2$	$d$	$d^2$	$d$	$d^2$	$d$	$d^2$	$d$	$d^2$
13.0	169	—21.2	449.44	—104.0	10816	—8.2	67.24	—112.2	12588.84	—125.2	15675.04
—248.0	61504	199.8	39920.04	44.0	1936	—48.2	2323.24	—4.2	17.64	243.8	59438.44
11.0	121	31.8	1011.24	34.0	1156	42.8	1831.84	76.8	5898.24	65.8	4329.64
73.0	5329	—35.2	1239.04	—7.0	49	37.8	1428.84	30.8	948.64	—42.2	1780.84
—7.0	49	—13.2	174.24	—41.0	1681	—20.2	408.04	—61.2	3745.44	—54.2	2937.64
—107.0	11449	65.8	4329.64	108.0	11664	—41.2	1697.44	66.8	4462.24	173.8	30206.44
38.0	1444	—48.2	2323.24	124.0	15376	—10.2	104.04	113.8	12950.44	75.8	5745.64
151.0	22801	—130.2	16952.04	33.0	1089	20.8	432.64	53.8	2894.44	—97.2	9447.84
96.0	9216	—119.2	14208.64	9.0	81	—23.2	538.24	—14.2	201.64	—110.2	12144.04
184.0	33856	5.8	33.64	19.0	361	189.8	36024.24	208.8	43597.44	24.8	615.04
—32.0	1024	—62.2	3868.84	46.0	2116	—94.2	8873.64	—48.2	2323.24	—16.2	262.44
96.0	9216	—288.2	83059.24	129.0	16641	—192.2	36940.84	—63.2	3994.24	—159.2	25344.64
—22.0	484	45.8	2097.64	—77.0	5929	23.8	566.44	—53.2	2830.24	—31.2	973.44
—37.0	1369	64.8	4199.04	—43.0	1849	27.8	772.84	—15.2	231.04	21.8	475.24
—223.0	49729	198.8	39521.44	19.0	361	—24.2	585.64	—5.2	27.04	217.8	47436.84
—35.0	1225	36.8	1354.24	—52.0	2704	1.8	3.24	—50.2	2520.04	—15.2	231.04
86.0	7396	—47.2	2227.84	—46.0	2116	38.8	1505.44	—7.2	51.84	—93.2	8686.24
—73.0	5329	41.8	1747.24	—91.0	8281	—31.2	973.44	—122.2	14932.84	—49.2	2420.64
2.0	4	47.8	2284.84	37.0	1369	49.8	2480.04	86.8	7534.24	84.8	7191.04
—30.0	900	—150.2	22560.04	146.0	21316	—180.2	32472.04	—34.2	1169.64	—4.2	17.64
—27.0	729	86.8	7534.24	56.0	3136	59.8	3576.04	115.8	13409.64	142.8	20391.84
—203.0	41209	148.2	21963.24	—172.0	29584	54.8	3003.04	—117.2	13735.84	—320.2	102528.04
—48.0	2304	117.8	13876.84	—45.0	2025	69.8	4872.04	24.8	615.04	72.8	5299.84
16.0	256	29.8	888.04	—38.0	1444	45.8	2097.64	7.8	60.84	—8.2	67.24
—79.0	6241	88.8	7885.04	—88.0	7744	9.8	96.04	—78.2	6115.24	0.8	0.64
TOTAL	273353		295709.40		150824		143674.00		156856.00		363647.40

$$\Sigma d^2 = 273353.00 + 295709.40 + 150824.00 + 143674.00 + 156856.00 \\ + 363647.40 = 1384063.80$$

$$\sigma d = \sqrt{\frac{1384063.80}{150}} = 96.06$$

$$E_d = \frac{\sigma d}{\sqrt{n}} = \frac{96.06}{5} = 19.212$$

The "significance" of the difference between any two types is given by the ratio of the difference of their means to the standard error (S. E.). The ratio for each comparison is as follows :—

$$A \text{ and } C = \frac{314.7 - 124.5}{19.212} = \frac{190.2}{19.212} = 9.90$$

$$B \text{ and } C = \frac{260.7 - 124.5}{19.212} = \frac{136.2}{19.212} = 7.09$$

$$D \text{ and } C = \frac{276.5 - 124.5}{19.212} = \frac{152.0}{19.212} = 7.91$$

$$A \text{ and } B = \frac{314.7 - 260.7}{19.212} = \frac{54.0}{19.212} = 2.81$$

$$B \text{ and } D = \frac{260.7 - 276.5}{19.212} = \frac{-15.8}{19.212} = -0.82$$

$$A \text{ and } D = \frac{314.7 - 276.5}{19.212} = \frac{38.2}{19.212} = 1.99$$

When this ratio is greater than 2.1, the chances of superiority of one type over the other are over 30 : 1.

Thus the superiority of varieties *A*, *D*, and *B* over *C*, and of *A* over *B* are statistically significant in the order given, according to the magnitude of the ratio, but the differences between *A* and *D*, and *B* and *D*, are not significant as the ratios of the mean difference to the standard error of the experiment are only 1.99 and —0.82 respectively and are much less than the expected ratio of 2.1.

In the original paper [Shaw and Bose, 1929] on yield trials with barley these results are also tested by Bessel's method and by Student's 'z'.

#### A YIELD TRIAL BY THE METHOD OF RANDOMIZED BLOCKS

##### *Example 20*

*Purpose of experiment.*—Varietal trial with gram.

*Field.*—Botanical Section, No. 6.

*Varieties.*—*A* (control), *B*, *C* and *D*.

*Lay-out.*—Randomized blocks.

*No. of replications.*—10.

*Size of plot.*—130' × 16' = 2,080 sq. ft.

Size of ultimate plots after cutting out the necessary borders.— $125' \times 12' = 0.0367$  acre.

Block Plot No. Type Yield in lb.

I	1	C	76.0
	2	A	51.0
	3	B	44.5
	4	C	67.5
	5	D	59.0
II	6	B	47.0
	7	D	74.0
	8	C	77.0
	9	A	57.5
III	10	C	80.0
	11	A	64.5
	12	B	43.0
	13	D	73.0
IV	14	A	67.5
	15	D	71.5
	16	B	48.5
	17	C	82.0
V	18	D	84.5
	19	A	78.0
	20	C	85.0
	21	B	40.0
VI	22	C	77.0
	23	D	67.0
	24	A	71.5
	25	B	49.0
VII	26	B	58.5
	27	C	87.5
	28	A	65.5
	29	D	74.0
VIII	30	C	94.5
	31	B	65.5
	32	D	83.5
	33	A	80.5
IX	34	D	82.0
	35	C	101.0
	36	B	77.0
	37	A	91.0
X	38	C	81.0
	39	A	63.5
	40	D	74.0
	41	B	64.5
	42	C	57.5

Fig. 18.—Plan of yield trial with randomized blocks (Example 20)

TABLE XXVII

*Calculation of differences and their squares (Example 20)*

Block	DIFFERENCES FROM ASSUMED MEAN OF 70 lb. PER PLOT				Block TOTALS	Squares of block totals
	A	B	C	D		
1	2	3	4	5	6	7
1	—19.0	—25.5	—2.5	—11.0	—58.0	3364.00
2	—12.5	—23.0	+7.0	+4.0	—24.5	600.25
3	—5.5	—27.0	+10.0	+3.0	—19.5	380.25
4	—2.5	—21.5	+12.0	+1.5	—10.5	110.25
5	+8.0	—30.0	+15.0	+14.5	+7.5	56.25
6	+1.5	—21.0	+7.0	—3.0	—15.5	240.25
7	—4.5	—11.5	+17.5	+4.0	+5.5	30.25
8	+10.5	—4.5	+24.5	+13.5	+44.0	1936.00
9	+21.0	+7.0	+31.0	+12.5	+71.0	5041.00
10	—6.5	—5.5	+11.0	+4.0	+3.0	9.00
VARIETAL TOTALS	—9.5	—162.5	+132.5	+42.5	+3.0 General total.	11767.50
Squares of varic- tal totals.	90.25	26406.25	17556.25	1806.25	Summation of squares of varietal totals. ..	45859.00

Total sum of squares of all differences in columns 2 to 5 = 8464.5

In the above table, differences in the yields of each plot from an assumed general mean of 70 lb. are set up for each variety in each plot. These differences are then added up horizontally to obtain total differences per block and vertically to get the total differences per variety. These totals are then squared and summed up so as to yield total sums of squares for blocks and for varieties. As these differences are taken from an assumed and not from the true mean, a correction is to be applied. This correction factor is obtained by squaring the general total at the bottom of column 6 (+ 3.0 in this case) and dividing it by the total number of plots in the experiment. To obtain true sums of squares due to (1) blocks, (2) varieties and (3) the total of the whole experiment, the correction factor must always be deducted from the crude sums. The total sum of squares can be obtained by squaring each individual difference and summing them together. So that we get:—

$$\text{Correction factor} = \frac{(3)^2}{40} = 0.225$$

$$\begin{array}{l} \text{Sum of squares due to blocks (divided by} \\ \text{the number of varieties)} \end{array} = \frac{11767.50}{4} - 0.225 = 2941.650.$$

$$\begin{array}{l} \text{Sum of squares due to varieties (divided by} \\ \text{the number of blocks)} \end{array} = \frac{45859}{10} - 0.225 = 4585.675.$$

$$\text{Total sum of squares} = 8464.500 - 0.225 = 8464.275.$$

### ANALYSIS OF VARIANCE

The figures obtained above are now set up in a table of analysis of variance and divided by their appropriate number of degrees of freedom so as to yield a measure of variance (mean square) for each item. Since there are 40 plots to be considered there are 40—1 or 39 total degrees of freedom. There being 10 blocks and 4 varieties in the experiment the degrees of freedom for blocks is 10—1 or 9 and for varieties it is 4—1 or 3. The degrees of freedom unaccounted for must be due to errors and are in this case 39—(9 + 3) = 27. The sum of squares due to error = total sum of squares — (sum of squares due to blocks + sum of squares due to varieties).

TABLE XXVIII  
*Analysis of Variance*

Due to				Degrees of freedom	Sum of squares	Mean square or variance
Blocks	.	.	.	9	2941.650	326.850
Varieties	.	.	.	3	4585.675	1528.558..... $V_1$
Error	.	.	.	27	936.950	34 702..... $V_2$
TOTAL				39	8464.275	

## SIGNIFICANCE

The above table of analysis of variance expresses the total variance divided into three different components. In the first instance we have the variance due to blocks. Since each block contains the same 4 varieties and is of the same size the variability of the yields of blocks is an expression of the variation in fertility (soil-heterogeneity) in the experimental field. The variance due to varieties, on the other hand, should be an expression of the inherent differences in yielding power between varieties; since each variety is distributed at random about the field in 10 different plots, this random distribution is designed to neutralize the effects of soil-heterogeneity. The balance left after deducting the variance due to blocks and the variance due to varieties from the total variance of the experiment is the variance due to the chance errors of experiment, and this latter quantity furnishes a criterion for measuring the significance of the experimental results. If the variance due to varieties is significantly greater than the variance due to error or blocks, then obviously the inherent differences between the yielding powers of the varieties has been the dominating factor in the experiment. If the variance due to blocks is greater than that due to varieties then the chief variable in the experiment has been the soil-heterogeneity. If the variance due to error is about equal to or larger than the variance due to varieties then the differences between the yielding powers of varieties have not been significant.

Bearing this explanation in mind it can easily be understood that the comparison of the variance due to varieties with that due to error, in other words, the ratio  $\frac{\text{Variance}_1}{\text{Variance}_2}$  will determine the significance of the experiment.

## FISHER'S 'z' TEST

Fisher estimates significance by taking half the logarithms to the base  $e$  (see appendix I) of the variances which are to be compared and subtracting the value of half  $\log_e$  for error from that for treatments. Thus in this experiment

$$\frac{1}{2} \log_e V_1 = \frac{1}{2} \log_e 1528.558 = \frac{7.3321}{2} = 3.6660$$

$$\frac{1}{2} \log_e V_2 = \frac{1}{2} \log_e 34.702 = \frac{3.5467}{2} = 1.7733$$

$$\text{Difference} = 1.8927$$

By taking  $\frac{1}{2} \log_e$ , we, of course, obtain the logarithm to the base  $e$  of the square root of the variance, that is of the standard deviation. The number thus obtained is now compared with the value of 'z' in Fisher's Table of 'z' for the appropriate degrees of freedom. Fisher's Table of 'z' gives the <sup>value</sup> ~~distribution~~ of the  <sup>$\frac{1}{2} \log_e V_1$</sup>  ~~ratio~~  $\frac{V_1}{V_2}$

at the  $P = 0.01$  and  $P = 0.05$  levels of significance; Fisher's 'z' must carefully be distinguished from Student's 'z'. In the present example the degrees of freedom for varieties are 3 and for error are 27. Entering the 'z' table on the 1 per cent

level of significance with  $n_1 = 3$  and  $n_2 = 27$ , we find  $z = 0.7631$ . The calculated value of 'z' is, however, 1.8927 which is much higher than the value required (0.7631) for significance at the 1 per cent level. We infer, therefore, that the differences in the yielding powers of varieties are of such a size as would not occur due to chance alone once in a hundred trials.

### MAHALANOBIS' 'x' TEST

With a view to simplifying calculations and avoiding the use of logarithms to the base  $e$ , Mahalanobis has published auxiliary tables of Fisher's 'z' in which the ratio  $\frac{V_1}{V_2} = \frac{s_1^2}{s_2^2}$  has been determined for degrees of freedom up to  $n = 60$  and  $n = \infty$ , and is called  $x$ . In our example,

$$x = \frac{V_1}{V_2} = \frac{1528.558}{34.702} = 44.05$$

The expected value of  $x$  from Mahalanobis' Tables is 5.488 for  $n_1 = 3$  and  $n_2 = 27$  for the 1 per cent level of significance.

Our observed value of  $X = 44.05$  being much greater than the expected value of 5.488, we can definitely conclude that the variance due to the varieties is significant and that the four varieties in the experiment show significant differences in their yielding powers.

### CRITICAL DIFFERENCE

To obtain an idea of the comparative yielding power of each variety, we prepare a table of differences of mean yields and determine which of the differences are greater or less than the critical difference of the experiment.

The critical difference is determined from Fisher's formula,  $t = \frac{d}{S.E.}$ , from which  $d = t \times S.E.$

The standard error of the difference can then be determined from the variance due to residual errors (see Table XXVIII) and the value of "t" for any level of probability appropriate to the degrees of freedom for error can be obtained from the table of "t". The critical difference is obtained from the product of these two values.

The standard error of the difference is calculated as follows:—

Variance due to error = 34.702

$$\therefore \text{Variance for mean values of 10 replications} = \frac{34.702}{10}$$

and the standard error of the difference between any two such values

$$= \sqrt{\frac{34.702 \times 2}{10}} = 2.634$$

Now for 27 degrees of freedom the values of “*t*” in Fisher’s Table are :—

2.771 for 0.01 level of significance

and 2.052 for 0.05 level of significance.

∴ The critical value, *d*, in this experiment

=  $2.771 \times 2.634$  or 7.2988 for 0.01 level of significance

and  $2.052 \times 2.634$  or 5.4050 for 0.05 level of significance.

TABLE XXIX

*Table of differences between mean yields of varieties in lbs.*

Varieties	A (control)	B	C	D	Mean yields in lb.	Percentage difference of mean yields from control (A)
1	2	3	4	5	6	7
A . .	..	—15.30	+14.20	+5.20	69.05	Percentage. ..
B . .	+15.30	..	+29.50	+20.50	53.75	—22.16
C . .	—14.20	—29.50	..	—9.00	83.25	+20.56
D . .	—5.20	—20.50	+9.00	..	74.25	+7.53
Rank in yielding power	III	IV	I	II	..	..

Positive differences which are statistically significant at  $P = 0.01$  level are printed in antique.

Critical difference at  $P = 0.01$  level . . . . . = 7.2988 ..

Critical difference at  $P = 0.01$  level as percentage of mean yield

of A or  $\frac{7.2988 \times 100}{69.05}$  . . . . . = 10.57 per cent.

In the last column (7) percentage differences larger than 10.57 are significant.

In the above table differences between the yields of any two varieties which are as large as or larger than the critical difference should be taken to be statistically significant. For the 1 per cent and the 5 per cent levels of significance, the critical differences in this example are 7.2988 and 5.4050 respectively. So that a difference as large as 5.405 lbs. or larger will occur five times in 100 trials as the result of chance errors of experiment and a difference of 7.2988 or larger will



occur only once in 100 trials as the result of chance errors. Statisticians have adopted these two levels of significance, on the ground that if a particular event occurs, by chance, only five times in a hundred trials, it may be expected that in the remaining 95 cases, the event will occur due to the inherent property of the variate under consideration. Similarly a more rigid test is the 1 per cent level which shows that the event may occur only *once* in a hundred trials by chance and in the remaining 99 trials it will occur because of an inherent property of the treatment to give such a result.

For the sake of convenience, positive differences greater than the critical difference may be underlined to show that these differences are statistically significant. In the present case it will be noted that the mean yield of variety *C* is significantly superior to the mean yields of all other varieties even at the 1 per cent level. Similarly the mean yield of variety *B* is significantly inferior to the yields of all other varieties at the 1 per cent level of significance. On the other hand, the mean yield of variety *D* is significantly superior to variety *B* and inferior to variety *C*, and although the yield of this variety is higher than that of variety *A*, the difference of 5.20 lbs. in their mean yields is not statistically significant.

The mean yields of each variety are ranked according to their comparative merits. In this experiment Type *A* was an old established strain [Type 17] of known yielding power and the main object was to determine whether any of the other types were superior or inferior to Type *A*.

The differences between each type and Type *A*, the control, may be conveniently expressed as percentages of the mean yield of the control and compared with the critical difference also expressed as a percentage of the mean yield of the control. This has been done in column 7 of Table XXIX.

Fisher's  $z$  and Mahalanobis'  $x$  are tests of significance of an experiment as a whole and before proceeding to make comparisons of individual yields some such test of significance must be applied. A test of significance in an experiment tells us whether the variance produced by treatments is significantly higher than the variance due to residual error. It may happen that mean differences greater than the critical difference will occur in experiments which are not indicated as significant by the  $z$  or  $x$  tests, and Fisher and Wishart [1930] warn workers that such mean differences are not to be taken as significant.

## A YIELD TRIAL BY THE LATIN SQUARE METHOD

### *Example 21*

*Purpose of experiment.*—Preliminary varietal trial with wheat.

*Field.*—Botanical Section, No. 10.

*Varieties.*—A, B, C, D, E, F, G, and H (control).

*Lay-out.*—Latin square,  $8 \times 8$ .

*Size of plots.*— $18' \times 18' = 324$  sq. ft.

*Size of ultimate plots after removing the necessary borders.  $14' \times 14' = 196$*

*sq. ft. =  $\frac{1}{222}$  acre approximately.*

The plots in this experiment are small owing to the number of types which had to be handled at this stage of the plant-breeding work on wheat; the example, however, serves to illustrate the statistical principles involved.

← COLUMNS →								
↑ ROWS ↓	C 121	D 80	E 89	A 90	F 87	B 81	H 88	G 65
	A 82	H 74	C 136	G 76	B 79	F 74	D 66	E 75
	D 96	F 78	B 56	E 74	H 86	A 90	G 68	C 100
	F 84	E 94	A 88	C 148	D 113	G 75	B 70	H 109
	H 75	G 69	D 96	B 89	A 108	C 143	E 103	F 97
	G 63	A 67	H 68	D 125	C 156	E 86	F 84	B 88
	B 59	C 130	G 71	F 80	E 100	H 85	A 91	D 112
	E 87	B 74	F 68	H 81	G 87	D 99	C 136	A 104

*Fig. 19.—Plan of wheat yield trial in a Latin Square.*

It will be noted that each variety has been so randomized as to occur once only in the direction of the rows and once only in that of the columns. The yield per plot is recorded in ounces.

Table XXX shows the deviations of plot yields from an assumed mean of 90 ounces. An assumed mean is taken in order to avoid big decimal figures and a final correction for this is made later on.

TABLE XXX

*Deviations of plot yields from an assumed mean of 90 oz.*

Rows				COLUMNS								Total deviation for rows
				1	2	3	4	5	6	7	8	
1	.	.	.	+31	-10	-1	0	-3	-9	-2	-25	-19
2	.	.	.	-8	-16	+46	-14	-11	-16	-24	-15	-58
3	.	.	.	+6	-12	-34	-16	-4	0	-22	+10	-72
4	.	.	.	-6	+4	-2	+58	+23	-15	-20	+19	+61
5	.	.	.	-15	-21	+6	-1	+18	+53	+13	+7	+60
6	.	.	.	-27	-23	-22	+35	+66	-4	-6	-2	+17
7	.	.	.	-31	+40	-19	-10	+10	-5	+1	+22	+8
8	.	.	.	-3	-16	-22	-9	-3	+9	+46	+14	+16
Total deviations for columns				-53	-54	-48	+43	+96	+13	-14	+30	+13 General total

To obtain an estimate of the total variance in the experiment the deviations of plot yields are squared and summed together as shown below :—

TABLE XXXI

*Squares of deviations given in Table XXX*

Rows				COLUMNS								Total sum of squares
				1	2	3	4	5	6	7	8	
1	.	.	.	961	100	1	0	9	81	4	625	1781
2	.	.	.	64	256	2116	196	121	256	576	225	3810
3	.	.	.	36	144	1156	256	16	0	484	100	2192
4	.	.	.	36	16	4	3364	529	225	400	361	4935
5	.	.	.	225	441	36	1	324	2809	169	49	4054
6	.	.	.	729	529	484	1225	4356	16	36	4	7379
7	.	.	.	961	1600	361	100	100	25	1	484	3632
8	.	.	.	9	256	484	81	9	81	2116	196	3232
Total sum of squares				3021	3342	4642	5223	5464	3493	3786	2044	General total sum of squares 31015

Total sum of squares from assumed mean = 31015  
 $\therefore$  " " " " " " true mean = 31015 — correction factor\*  
 = 31015 — 2.64 = 31012.36

\* See page 110.

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Similarly the deviations of plot yields for each variety are summed up for the replications per variety and tabulated as shown below :—

TABLE XXXII

*Deviation of plot yields for each variety from assumed mean*

Replications	VARIETIES								Total deviations for rows
	A	B	C	D	E	F	G	H	
1 . .	0	—9	+31	—10	—1	—3	—25	—2	—19
2 . .	—8	—11	+46	—24	—15	—16	—14	—16	58
3 . .	0	—34	+10	+6	—16	—12	—22	—4	—72
4 . .	—2	—20	+58	+23	+4	—6	—15	+19	+61
5 . .	+18	—1	+53	+6	+13	+7	—21	—15	+60
6 . .	—23	—2	+66	+35	—4	—6	—27	—22	+17
7 . .	+1	—31	+40	+22	+10	—10	—19	—5	+8
8 . .	+14	—16	+46	+9	—3	—22	—3	—9	+16
Total deviation for varieties	0	—124	+350	+67	—12	—68	—146	—54	+13 General total.
Mean deviation	0	—15.50	+43.75	+8.375	—1.50	—8.50	—18.25	—6.75	..
Mean yield	90.00	74.50	133.75	98.38	88.50	81.50	71.75	83.25	..

Now the sums of squares for rows, columns and varieties are calculated as shown below :—

TABLE XXXIII

*Sum of squares of deviations for rows, columns and varieties*

Replications					Rows		Columns		Varieties	
					<i>d</i>	<i>d</i> <sup>2</sup>	<i>d</i>	<i>d</i> <sup>2</sup>	<i>d</i>	<i>d</i> <sup>2</sup>
1	.	.	.	.	—19	361	—53	2809	0	0
2	.	.	.	.	—58	3364	—54	2916	—124	15367
3	.	.	.	.	—72	5184	—48	2304	+350	122500
4	.	.	.	.	+61	3721	+43	1849	+67	4489
5	.	.	.	.	+60	3600	+96	9216	—12	144
6	.	.	.	.	+17	289	+13	169	—68	4624
7	.	.	.	.	+8	64	—14	196	—146	21316
8	.	.	.	.	+16	256	+30	900	—54	2916
TOTAL .					..	16839	..	20359	..	171365
Divided by 8 to get mean values					..	2104.88	..	2544.88	..	21420.62
Subtract correction . .					..	2.64	..	2.64	..	2.64
True sum of squares . .					..	2102.24	..	2542.24	..	21417.98

The total deviations for rows, columns and varieties are obtained by squaring each deviation and summing. These totals are then divided by the number of replications and we get average sums of squares. As we arbitrarily took an assumed mean of 90 oz. a correction has now to be applied to obtain the true sums of squares. This correction is equal to the square of the general total [Table XXXII] divided by the number of plots in the experiment. Thus—

$$\text{Correction factor} = \frac{(13)^2}{64} = 2.64$$

#### ANALYSIS OF VARIANCE

Now the most important table, that of the analysis of variance, has to be set up. This table gives the complete information that can be gathered from the experiment. The total variation in the experiment can be divided into four parts in which there are 64 — 1 or 63 degrees of freedom. For rows, columns, and varieties each there are 8 — 1 = 7 degrees of freedom. The degrees of freedom for error = total degrees of freedom — (sum of degrees of freedom for rows, columns and varieties). In this case, it is 63 — (7 + 7 + 7) = 42. Similarly the sum of squares for error is equal to the difference between the total sum of squares and the summation of the sums of squares for rows, columns, and varieties. The mean squares or variance for each of these groups of variables can be determined by dividing the sums of squares by their appropriate degrees of freedom,

TABLE XXXIV  
*Analysis of variance*

Due to	Degrees of freedom	Sum of squares	Mean squares of variance	$\frac{1}{2} \log.e$ Mean squares for Fisher's $z$ -test	Ratio of $\frac{v_1}{v_2}$ for Mahalanobis' $x$ -test
1	2	3	4	5	6
Rows . . . .	7	2102.24	300.32	2.85236	..
Columns . . . .	7	2542.24	363.18	2.94748	..
Varieties . . . .	7	21417.98	3059.71( $v_1$ )	4.01337	..
Error . . . .	42	4949.90	117.85( $v_2$ )	2.3848	$\frac{3059.71}{117.85}$ = 25.963
TOTAL .	63	31012.36	..	..	..

Columns 1 to 4 in the above table are essential and either column 5 or 6 may be retained according to whether the significance of the result is to be determined by Fisher's original  $z$ -test or by its auxiliary-test, the  $x$ -test, recently brought out by Mahalanobis.

#### SIGNIFICANCE

The same argument which was followed in the case of the randomized blocks is now used in the interpretation of the Latin Square. If the mean square or variance due to varieties or treatments is much greater than the mean square due to "error" it may be concluded that the differences between the varieties or treatments are significant. Similarly if the variance due to rows or to columns be very much greater than the variance due to "error", large variations in soil-fertility are obviously present. The advantage of the Latin Square lay-out lies in the fact that it affords an elimination of soil-heterogeneity in two directions at right angles to each other, *i.e.*, in rows and in columns. In practice, therefore, we compare the variance ( $v_1$ ) due to treatments with the variance ( $v_2$ ) due to error. This gives a more precise measure of the significance of the treatments than the variance of the whole experiment since the variability due to differences in soil fertility has been eliminated.

#### FISHER'S $z$ -TEST

In our example,  $n_1 = 7$  and  $n_2 = 42$  and the observed value of  $z = 4.01337 - 2.3848 = 1.6285$ ; the value of  $z$  from Fisher's tables for  $n_1 = 6$  and  $n_2 = 30$  (near about the true degrees of freedom)\* is

0.6226 for the 1 per cent level ( $P = 0.01$ )  
and 0.4420 ,, ,, 5 ,, ,, ,, ( $P = 0.05$ ).

\* Fisher's  $z$ -table ends at  $n_2 = 30$ ; by taking  $n_2 = 30$  we are adopting an even stricter criterion than if we took the actual value of  $z$  for  $n_2 = 42$ .

The observed value being greater than the stricter criterion at the 1 per cent level it is concluded that there is a statistical difference between the different varieties in the experiment.

### MAHALANOBIS' $x$ -TEST

$$\text{Observed value of } x = \frac{v_1}{v_2} = \frac{3059.71}{117.85} = 25.963$$

For  $n_1 = 6$  and  $n_2 = 30$

Expected value of  $x = 3.474$  for  $P = 0.01$

and  $x = 2.421$  for  $P = 0.05$

The observed ratio of 25.963 being greater than the expected ratio of 3.474 at the  $P = 0.01$  level it is concluded that the differences between the varieties are statistically significant.

Having established that there are significant differences in the yielding powers of different varieties, the varieties may be compared amongst themselves. This, of course, is done by comparing the mean differences with the critical difference of the experiment. Details regarding the calculation of the critical difference as well as the tabulation of differences in the mean yields of varieties have been given in the case of randomized blocks and need not be repeated here. In this case we get—

Variance due to error = 117.85

$$\therefore \text{Variance for mean values of 8 replications} = \frac{117.85}{8}$$

and standard error of difference between any two such values =  $\sqrt{\frac{117.85 \times 2}{8}} = 5.43$ .

The value of “ $t$ ” for 42 degrees of freedom is not given in Fisher’s “ $t$ ”-table but can easily be interpolated, or by taking the value given for 30 degrees of freedom we can work with a stricter criterion.

For 30 degrees of freedom the value of “ $t$ ” in Fisher’s Tables is

2.750 for 0.01 level of significance and 2.042 for 0.05 level of significance.

$\therefore$  The critical value  $d = t \times S. E. = 2.750 \times 5.43 = 14.9325$  for 0.01 level of significance

and  $d = 2.042 \times 5.43 = 11.0880$  for 0.05 level of significance.

Differences greater than these two critical differences for the 0.01 and 0.05 levels in the table shown below are statistically significant. Those which fall below 11.0880 must be considered as not significant.

TABLE XXXV

*Table of differences between mean yields of varieties in oz.*

Varieties	A	B	C	D	E	F	G	H (control)	Mean yields in oz.	Percentage difference of mean yields from control (H)
A . . . . .	..	-15.50	+43.75	+8.375	-1.50	-8.50	-18.25	-6.75	90.00	% +8.11
B . . . . .	+15.50	..	+59.25	+23.87	+14.00	+7.00	-2.75	+8.75	74.50	-10.51
C . . . . .	-43.75	-59.25	..	-35.37	-45.25	-52.25	-62.00	-50.50	133.75	+60.66
D . . . . .	-8.37	-23.87	+35.37	..	-9.87	-16.87	-26.62	-15.12	98.88	+18.17
E . . . . .	+15.50	-14.00	+45.25	+9.875	..	-7.00	-16.75	-5.25	88.50	+6.31
F . . . . .	+8.50	-7.00	+52.25	+16.87	+7.00	..	-9.75	+1.75	81.50	-2.10
G . . . . .	+18.25	+2.75	+62.00	+26.62	+16.75	+9.75	..	+11.50	71.75	-13.82
H . . . . .	+6.75	-8.75	+50.50	+15.12	+5.25	-1.75	-11.50	..	83.25	..
Rank in yielding power	III	VII	I	II	IV	VI	VIII	V	..	..

Critical difference at  $P = 0.01$  level

14.9325

Critical difference at  $P = 0.01$  level as percentage of mean yield of H,  $\frac{14.9325 \times 100}{83.25}$ 

= 17.937 per cent.



In the last column percentage differences greater than 17.937 are significant.

In the table, differences greater than critical difference for 1 per cent level are marked in antiques and differences greater than critical difference for 5 per cent level are marked in italics. The results are evident from a mere inspection of the above table.

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## CHAPTER X

## STATISTICAL INTERPRETATION OF COMPLEX AND SERIAL EXPERIMENTS

## COMPLEX EXPERIMENTS

The experiments detailed above and their interpretation by Fisher's analysis of variance are confined to the investigation of only one variable factor. In examples 20 and 21 we are concerned with the study of the yielding power of a few varieties of gram and wheat, and in such experiments all the factors except one, the kind of seed sown, are the same for the different plots. The land for such an experiment is so chosen that the soil variability is as small as possible and all plots are cultivated in the same manner. In short, within the limits of field conditions, the different plots of the experiment have only one changing factor; such an experiment, in which only one variable changes, is termed a simple experiment. It sometimes happens that it is desirable to design an experiment to test two, three or more variable factors. In such cases if we resort to the simple method we have to perform a number of experiments each like the one detailed in example 20 or 21. The space, the amount of labour and the expense involved in carrying out these experiments are not commensurate with the advantage gained and to avoid such difficulties, a complex experiment either a Latin Square or a randomized block, involving the number of factors in question is laid out. Such an experiment in which we are concerned with the study of a number of factors simultaneously is known as a complex experiment.

An elementary example of a complex experiment is furnished by the lay-out of a field trial involving three varieties,  $C_1$ ,  $C_2$ , and  $C_3$ , and three manures  $M_1$ ,  $M_2$ , and  $M_3$ . The nine possible combinations resulting from the use of three varieties and three manures are shown below and can be arranged in a  $9 \times 9$  Latin Square :—

Arrangement Number	Variety		Manure
1	$C_1$	and	$M_1$
2	$C_2$	„	$M_1$
3	$C_2$	„	$M_2$
4	$C_3$	„	$M_2$
5	$C_1$	„	$M_2$
6	$C_3$	„	$M_3$
7	$C_3$	„	$M_1$
8	$C_2$	„	$M_3$
9	$C_1$	„	$M_3$

The arrangement of treatments in the Latin Square can be as follows :—

TABLE XXXVI

3	9	2	5	1	8	4	7	6
4	5	7	6	8	2	1	9	3
8	7	1	2	6	4	9	3	5
1	2	3	7	4	9	6	5	8
7	4	8	3	5	1	2	6	9
2	1	6	9	3	5	7	8	4
5	3	9	1	7	6	8	4	2
9	6	4	8	2	3	5	1	7
6	8	5	4	9	7	3	2	1

In this Latin Square each variety occurs once with each manure in each row and in each column. In this example there are only two factors under consideration. Now if we have  $n_1$  types of wheat,  $n_2$  manures and  $n_3$  methods of cultivation we proceed just as in the previous case, but there will then be  $n_1 \times n_2 \times n_3$  combinations and these would be arranged in a  $(n_1 \times n_2 \times n_3)$  Latin Square. If the product  $n_1 \times n_2 \times n_3$  is large, the square of this becomes still larger, and consequently the size of the field required for such an experiment will be very large. In such cases it is better, therefore, to adopt the method of randomized blocks with about ten replications.

#### Example 22—

As an example of a complex experiment we may consider an experiment on the effect of four fungicides on the incidence of bunt (*Tilletia indica*) in three varieties of wheat using two methods of infection [Mitra, 1935]. In such an experiment there are three variables—fungicides, varieties of wheat and methods of infection.

The fungicides used were copper carbonate, ceresan, formalin and uspulun. These four fungicides together with an untreated control make up five treatments.

The three varieties of wheat were—

Pusa types 111, 112 and 113.

The two methods of infection were—

A—naturally infected seed, and

B—naturally infected seed plus a dose of artificial infection.

Further details of the mycological side of this experiment will be found in the author's original paper.

The lay-out was a randomized block with 8 replications. Each block contained 30 plots, 15 under infection series A and 15 under infection series B ; 5 treatments and 3 varieties in each series. At the time of harvest all the ears in each plot were counted and the number of ears showing bunt noted down. The amounts of bunt in each plot are given as percentages of the total number of ears in each plot and are shown in Table XXXVII.

TABLE XXXVII

*Percentage of bunt infection*

Block No.	Varieties	TREATMENTS															TOTALS		
		1. Control			2. Copper carbonate			3. Ceresan			4. Formalin			5. Usputum			Series A.	Series B.	For Blocks
		P111	P112	P113	P111	P112	P113	P111	P112	P113	P111	P112	P113	P111	P112	P113			
I	Infection Series A	0.94	1.54	3.33	.39	.55	.12	.19	.19	.22	2.22	.65	.42	.16	.41	1.33	13.38	..	32.46
		7.40	5.00	2.23	.32	.80	.72	.44	.66	.20	.00	.47	.32	.22	.00	.00	..	19.08	..
II	Infection Series B	0.80	1.67	2.89	.19	1.74	.34	.68	.76	.39	.66	1.08	.48	.19	.16	.41	12.44	..	29.40
		3.81	5.81	2.28	.13	.34	.28	.78	.84	.51	.00	.33	.27	.41	.28	.84	..	16.96	..
III	Infection Series A	0.85	5.33	3.18	.41	.98	.14	.35	1.93	.37	1.11	.69	.82	.15	.17	.24	16.72	..	37.73
		3.47	9.75	3.53	.17	.43	.27	.27	1.09	.11	.00	.00	.93	.55	.29	.15	..	21.01	..
IV	Infection Series B	0.84	2.90	3.07	.29	.87	.00	.24	1.57	.41	1.62	.63	1.12	.00	.78	.21	14.55	..	37.24
		3.75	10.20	2.44	.29	.44	.59	.40	.50	.14	.00	2.50	.44	.19	.40	.41	..	22.69	..
V	Infection Series A	1.64	1.92	2.83	.26	.89	.18	.18	1.22	.23	.84	.85	.44	.11	.27	.48	12.34	..	38.71
		5.89	8.18	4.79	.46	.37	.65	.35	1.17	.19	.89	1.38	.99	.00	.50	.62	..	26.37	..
VI	Infection Series B	0.56	2.66	1.34	.42	.47	.22	.24	.82	.15	.88	.86	.50	.12	.48	.32	10.04	..	32.20
		8.62	5.46	2.02	.23	.11	.09	.34	.24	.24	.60	1.53	1.06	.61	.22	.79	..	22.16	..
VII	Infection Series A	0.69	3.90	2.48	1.22	.58	.15	.55	.93	.47	1.25	.32	.21	.33	1.05	.57	14.70	..	28.90
		3.28	3.32	2.90	.32	.34	.16	.57	.86	.37	.00	1.00	.00	.34	.12	.62	..	14.20	..
VIII	Infection Series B	0.47	2.13	2.40	1.54	.56	.14	.16	1.37	.22	1.39	.72	1.40	.00	.18	.44	13.12	..	33.80
		3.02	6.25	3.19	.48	.61	.60	.49	.32	.42	.00	3.06	1.33	.21	.33	.27	..	20.68	..
TOTAL OF VARIETIES.	Series A.	6.79	22.05	21.52	4.72	6.64	1.29	2.59	9.51	2.46	9.97	5.80	5.39	1.06	3.50	4.00	107.29	..	..
	Series B.	39.24	53.97	23.38	2.39	3.44	3.36	3.64	5.98	2.18	1.49	10.37	5.34	2.53	2.14	3.70	..	163.15	..
TOTAL	A + B	46.03	76.02	44.90	7.11	10.08	4.65	6.23	15.49	4.64	11.46	16.17	10.73	3.59	5.64	7.70			270.44
TOTAL OF TREATMENTS		166.95			21.84			26.36			38.86			16.93					

The statistical analysis of the data is done by Fisher's analysis of variance. Here the total variance is analysed into variances due to blocks, infections, varieties, interactions between the combinations of the three factors and the residual variance. The crux of the problem is to find out the variances due to the several variables. The total sum of squares is calculated as in the ordinary simple case. The variable squared method of calculation being used, the correction factor to be applied is  $\frac{(270.44)^2}{240}$  or 304.740. The total sum of squares equals the sum of squares of the percentage infections of different plots minus the correction factor :—

$$(0.94)^2 + (0.80)^2 + (0.85)^2 + \dots + (0.27)^2 - \frac{(270.44)^2}{240}$$

$$= 938.896 - 304.740 = 634.156.$$

Since there are 30 observations in a block (15 under each method of infection) the sum of squares of the block totals is to be divided by 30 and therefore the sum of squares between blocks is equal to 1/30th of the sum of squares of the block totals (ignoring effects of variety and treatment) minus the correction factor. Thus,

$$1/30\text{th } (32.46)^2 + (29.40)^2 + (37.73)^2 + \dots + (33.8)^2 - 304.740$$

$$= \frac{9241.3362}{30} - 304.74 = 3.305.$$

The tables given below enable us to determine the sums of squares between treatments, varieties, infections and interactions between any two of these variables.

TABLE XXXVIII

*Treatments × Infections*

—	T. 1	T. 2	T. 3	T. 4	T. 5	TOTALS.
A	50.36	12.65	14.56	21.16	8.56	107.29
B	116.59	9.19	11.80	17.20	8.37	163.15
TOTAL	166.95	21.84	26.36	38.36	16.93	270.44

The first value, 50.36, in the first row and column is derived from the summation of the total percentage for 3 wheats under Treatment 1 (T.1), i.e., 6.79 + 22.05 + 21.52 = 50.36.

TABLE XXXIX

*Treatments × Varieties*

—	T. 1	T. 2	T. 3	T. 4	T. 5	TOTALS
P. 111	46.03	7.11	6.23	11.46	3.59	74.42
P. 112	76.02	10.08	15.49	16.17	5.64	123.40
P. 113	44.90	4.65	4.64	10.73	7.70	72.62
TOTAL	166.95	21.84	26.36	38.36	16.93	270.44

The figure 46.03 is the summation of all the percentage figures for P. 111 under control, (T. 1).

TABLE XL

*Varieties × Infections*

—	P. 111	P. 112	P. 113	TOTALS.
A . .	25.13	47.50	34.66	107.29
B . .	49.29	75.90	37.96	163.15
TOTAL .	74.42	123.40	72.62	270.44

The figure, 25.13, is derived from the summation of the percentage for P. 111 under all the treatments in Series A, *viz.*,

$$6.79 + 4.72 + 2.59 + 9.97 + 1.06 = 25.13$$

Sum of squares due to treatments

$$\begin{aligned}
 &= \frac{\text{Sum of squares of treatment totals}}{48 \text{ i.e., the number of plots for each treatment}} - C. F. (\text{correction factor}) \\
 &= \frac{(166.95)^2 + (21.84)^2 + (26.36)^2 + (38.36)^2 + (16.93)^2}{48} - 304.740 \\
 &= \frac{30802.2522}{48} - 304.740 = 336.974 \text{ with 4 degrees of freedom,}
 \end{aligned}$$

The sum of squares due to infections

$$\begin{aligned}
 &= \frac{\text{Sum of squares of infection totals}}{120 \text{ (i.e., number of plots for each infection)}} - C.F. \\
 &= \frac{(107.29)^2 + (163.15)^2}{120} - 304.740 \\
 &= 13.002, \text{ the degree of freedom being 1.}
 \end{aligned}$$

Interaction between treatments and infections is equal to the sum of squares due to treatments  $\times$  infections — (Sum of squares due to treatments + Sum of squares due to infections). Sum of squares for combined treatments  $\times$  infections =  $1/24 \{ (50.36)^2 + (12.65)^2 + (14.56)^2 + (21.16)^2 + (8.56)^2 + (116.59)^2 + (9.19)^2 + (11.80)^2 + (17.20)^2 + (8.37)^2 \} - 304.740 = 429.093$ .

Hence the interaction between treatments  $\times$  infections =  $429.093 - 336.974 - 13.002 = 79.117$  with  $(9-4-1 \text{ or } 4)$  degrees of freedom.

Tables XXXIX and XL enable us to calculate the sum of squares between varieties and also the interactions between varieties  $\times$  treatments and varieties  $\times$  infections.

Sum of squares between varieties

$$\begin{aligned}
 &= \frac{(74.42)^2 + (23.40)^2 + (72.62)^2}{80 \text{ (i.e., the number of plots)}} - 304.740 \\
 &= 20.7545 \text{ under each variety.}
 \end{aligned}$$

$$\begin{aligned}
 \text{Total sum of squares for treatments } \times \text{ varieties (ignoring infection and block effects)} &= \frac{(46.03)^2 + (7.11)^2 + \dots + (7.70)^2}{16 \text{ (i.e., 8 blocks with 2 infections)}} - 304.740 \\
 &= \frac{10999.746}{16} - 304.740 \text{ in each block} \\
 &= 382.747
 \end{aligned}$$

Interaction between treatments  $\times$  varieties = Total sum of squares — (Sum of squares for treatments + Sum of squares for varieties) having  $(14-4-1) = 8$  degrees of freedom, i.e., equal to  $382.747 - 20.7545 - 336.974 = 25.019$ .

To find interaction between varieties and infections, we make use of Table XL. Interaction between varieties and infections is equal to total sum of squares — (Sum of squares due to varieties + sum of squares due to infections).

Sum of squares for combined varieties  $\times$  infections

$$\begin{aligned}
 &= \frac{\{ (25.13)^2 + (47.50)^2 + (34.66)^2 + (49.29)^2 + (75.90)^2 + (37.96)^2 \}}{40 \text{ (i.e., 5 treatments and 8 blocks)}} - 304.740 \\
 &= 38.2675
 \end{aligned}$$

$$\begin{aligned}
 \text{Interaction} &= 38.2675 - 20.7545 - 13.002 \\
 &= 4.511 \text{ having } (5-2-1 \text{ or } 2) \text{ degrees of freedom.}
 \end{aligned}$$

Finally, there remains to be determined the interactions between the three variables.

This is equal to the total sum of squares (ignoring block effect) minus sum of variances due to treatments, varieties, infections and interactions between the three quantities taken two at a time, having (29—4—1—2—4—8—2 or 8) degrees of freedom.

Total sum of squares for treatments  $\times$  varieties  $\times$  infections (excluding block effect)

$$= \frac{(6.79)^2 + (22.05)^2 + (21.52)^2 + (4.72)^2 + \dots + (3.70)^2}{8 \text{ (i.e., number of blocks)}} - 304.740 = 520.612.$$

Therefore, interaction between treatments  $\times$  infections  $\times$  varieties  
 $= 520.612 - (336.974 + 13.002 + 20.755 + 79.117 + 25.019 + 4.51)$   
 $= 41.234$  with 8 degrees of freedom.

The difference between the total sum of squares and the sum of all the other sums of squares gives the residual, having (239—7—29 or 203) degrees of freedom.

The final analysis of variance may now be tabulated as follows :—

TABLE XLI  
*Analysis of Variance*

Due to	Degrees of freedom	Sum of squares	Mean Square	Observed value of Mahalanobis' $\chi$	Critical value of Mahalanobis' $\chi$ (P = 0.01)
Blocks . . . . .	7	3.305	0.472	..	..
Treatments (Control and fungicides) .	4	336.974	84.244	155.144	3.320
Infections (A & B) . . . . .	1	13.002	13.002	23.946	6.635
Varieties (Wheat types P. 111, P. 112 and P. 113).	2	20.755	10.378	19.111	4.605
Interactions :					
Treatments $\times$ Infections . . . . .	4	79.117	19.780	36.426	3.320
Treatments $\times$ Varieties . . . . .	8	25.019	3.127	5.760	2.511
Infections $\times$ Varieties . . . . .	2	4.511	2.255	4.153	4.605
Treatments $\times$ Varieties $\times$ Infections .	8	41.234	5.154	9.492	2.511
Residual error . . . . .	203	110.239	0.543	..	..
TOTAL .	239	634.156	..	..	..

It will be seen from the above table that the calculated value of  $\chi$  exceeds the theoretical value given in Mahalanobis' tables at 1 per cent level of significance in all items except blocks, showing thereby that the differences between the several treatments, infections and varieties are statistically significant. Further, it may be noted that the interactions between the various combinations of the three factors under consideration are also significant.

After having established that there are significant differences in percentage attack by bunt on treatments, infections and varieties, let us now compare the effect of the fungal attack within every one of the factors under discussion.



To compare and to arrange the five treatments, the two infections and the three varieties in order of merit in respect of bunt attack, we have first to calculate the critical differences for treatments, infections and varieties. Then a table of differences between the mean percentage of attack due to bunt is drawn up for every one of the factors under study.

With the help of the critical differences for the respective factors these tables will enable us to judge the merits of (1) the various treatments, (2) the different kinds of infections and (3) different varieties used for the experiment.

Table XLII gives the differences between the means of the different treatments, control, copper carbonate, ceresan, formalin and uspulun. The critical differences for treatments, etc., have been calculated and given below the respective tables.

TABLE XLII

*Table of differences between the mean values for treatments*

Treatments	T. 1	T. 2	T. 3	T. 4	T. 5
T. 1 . . . . .	..	— 3·023	— 2·929	— 2·679	— 3·125
T. 2 . . . . .	+ 3·023	..	+ 0·094	+ 0·344	— 0·102
T. 3 . . . . .	+ 2·929	— 0·094	..	+ 0·250	— 0·196
T. 4 . . . . .	+ 2·679	— 0·344	— 0·250	..	— 0·446
T. 5 . . . . .	+ 3·125	+ 0·102	+ 0·196	+ 0·446	..
Mean . . . . .	3·478	0·455	0·549	0·799	0·353

$$\text{Critical difference} = \sqrt{\frac{0.543 \times 2}{48}} \times t = 0.304 \quad (P = 0.05)$$

$$\text{,, ,,} \quad \quad \quad = 0.401 \quad (P = 0.01)$$

A comparison of the various figures of the table with the critical differences leads us to the following conclusions :—

That T. 1 (control) differs from all other treatments in having significantly higher percentage of bunt infection.

That T. 4 (formalin) has significantly higher infection than T. 2 (copper carbonate) at  $P = 0.05$  and T. 5 (uspulun) at the higher level,  $P = 0.01$ .

Or, in other words, we may conclude that

T. 5 (uspulun) is superior to T. 4 (formalin) at the 1 per cent level ;

T. 2 (copper carbonate) is superior to T. 4 (formalin) at the 5 per cent level ;

T. 2 (copper carbonate), T. 3 (ceresan), T. 4 (formalin) and T. 5 (uspulun) are superior to T. 1 (control or no disinfectant) at the 1 per cent level,

There is no significant difference between other combinations,

In Table XLIII, we get the percentage differences of bunt infection between the means of the different varieties P. 111, P. 112 and P. 113.

TABLE XLIII

*Table of differences between the mean values for varieties*

Varities	P. 111	P. 112	P. 113
P. 111 . . . . .	..	+ 0.613	— 0.022
P. 112 . . . . .	— 0.613	..	— 0.535
P. 113 . . . . .	+ 0.022	+ 0.535	..
Mean . . . . .	0.930	1.543	0.908

$$\text{Critical difference} = \sqrt{\frac{.545 \times 2}{80}} \times t = 0.229 (P = 0.05)$$

$$\text{,, ,,} \quad \quad \quad = 0.303 (P = 0.01)$$

From the table it is evident that P. 112 is inferior to P. 111 and P. 113 at the 1 per cent level in respect of susceptibility to bunt.

The comparison of infections is quite easy in this particular case, for we have only two kinds of infections.

Mean of A . . . . .	0.893
Mean of B . . . . .	1.360
Mean difference . . . . .	0.467

$$\text{Critical difference} = \sqrt{\frac{0.545 \times 2}{120}} \times t = 0.247 (P = 0.01),$$

which shows that infection is significantly higher in the B series than in the A series.

#### SERIAL EXPERIMENTS

In the case of complex experiments we have seen that an experiment can test the effect of more than one variable, such as the combined effect of different manures and different varieties. It has been previously mentioned that conclusions based upon a single experiment conducted in one season may not be supported when that experiment is repeated in another season or under different climatic conditions. It is quite possible, for instance, that different treatments may respond differentially when subjected to varying climatic conditions and for this reason a repetition of the experiment in at least three successive seasons is desirable. An investigation which is repeated in several successive seasons is termed a SERIAL EXPERIMENT and the accumulated results of such successive experiments are combined to give a more exact evaluation of the different treatments.

*Example 23.*—The oat trial at Pusa furnishes an excellent example of the utility of such accumulated data in performing a SERIAL TRIAL [Bose, 1936].

Eleven hybrid oats, *A, B, C, D, E, F, G, H, I, J* and *K*, which were evolved at Pusa by hybridizing Scotch Potato oats with two Indian oats [Shaw and Bose, 1933<sub>2</sub>], were selected for comparison with two established high-yielding Pusa selections, B. S. 1 (marked *L* in the following pages) and B. S. 2 (marked *M*). Details regarding B. S. 1 and 2 have already been published [Shaw and Bose, 1933<sub>1</sub>].

In a preliminary trial at Pusa conducted in 1930-31, small areas of equal sizes, under a large number of oats, were harvested from duplicate plots of each type. The oat hybrids and selections enumerated above showed great promise and in table 1, the average yields and the rank attained by each type are given.

TABLE XLIV

*Mean yields of Pusa oats obtained from duplicate plots each measuring 50' × 8'*

Yields and rank	VARIETIES												
	<i>A</i>	<i>B</i>	<i>C</i>	<i>D</i>	<i>E</i>	<i>F</i>	<i>G</i>	<i>H</i>	<i>I</i>	<i>J</i>	<i>K</i>	<i>L</i> (B. S. 1)	<i>M</i> (B. S. 2)
Yields in lb. .	11.55	13.05	15.36	15.15	15.35	14.55	13.90	12.75	12.80	13.85	12.90	11.75	11.45
Rank . .	12	7	1	3	2	4	5	10	9	6	8	11	13

In the following year, 1931-32, randomized blocks with five replications of each type were laid out simultaneously at Pusa and Karnal with these thirteen oats. The usual soil and cultural conditions required for oats in this country were given and soils of average fertility, without the application of any manures, were used. The main variable factor in the two localities was that at Pusa the crop was grown under *barani* conditions, i.e., without any irrigation, whereas at Karnal the crop usually received two to three irrigations. The yields of these thirteen types of oats taken from five plots, each 1,000 square feet in area, obtained at Pusa and at Karnal are recorded with their respective merits in the Table XLV.

TABLE XLV

*Total yields of Pusa oats taken from five plots, each 1,000 square feet in area (1931-32)*

Localities	Yields and rank	VARIETIES												
		<i>A</i>	<i>B</i>	<i>C</i>	<i>D</i>	<i>E</i>	<i>F</i>	<i>G</i>	<i>H</i>	<i>I</i>	<i>J</i>	<i>K</i>	<i>L</i>	<i>M</i>
Pusa	Yields in lb.	127.5	123.5	122.0	131.5	154.5	174.0	151.0	111.0	116.5	159.0	136.0	221.5	161.0
	Rank	9	10	11	8	5	2	6	13	12	4	7	1	3
Karnal	Yields in lb.	206.5	146.5	210.5	203.0	178.5	172.0	218.5	182.5	164.0	221.5	197.0	159.0	178.0
	Rank	4	13	3	5	8	10	2	7	11	1	6	12	9

By comparing Tables XLIV and XLV, it will be noted that the yielding powers of the different oats at Pusa in 1931-32 and 1930-31 were not at all similar. Whereas *L* (B. S. 1) ranked as the highest yielder in the latter year, it was indeed a poor yielder in the former season. Under Karnal conditions, however, this very type of oats ranked very low in 1931-32, a result which was in conformity with that obtained at Pusa in 1930-31. It is evident from Table XLV that if recommendations had been based on the results of the Pusa trial conducted during this single season, we should certainly speak very highly of *L* (B. S. 1) and reject hybrid C for its poor yields. If the recommendations, on the other hand, were based on Karnal results of 1931-32 we should form just the opposite view. This proves definitely how erroneous it would be to base conclusions on the experience of trials conducted in one season or in one locality.

This yield trial experiment was, therefore, repeated in these two localities in the *rabi* seasons of 1931-32, 1932-33, and 1933-34, and Table XLVI shows the yields per variety obtained from plots 1,000 square feet in area. The size of the plot varied somewhat from year to year, and from locality to locality, depending on the size of the field available for the experiment. To maintain uniformity the plot yields in the table under reference have been calculated on the basis of areas of 1,000 square feet, which is roughly equal to 1/44th of an acre.

It will be noted from the Table XLVI that the variables under consideration are :—

- (1) Localities—Pusa and Karnal.
- (2) Seasons—1931-32, 1932-33, and 1933-34.
- (3) Varieties—Hybrids A to K and types B. S. 1 (*L*) and B. S. 2 (*M*) oats.
- (4) Blocks—5 replications of each type.

The method of obtaining the analysis of variance is the same as is employed in the case of complex experiments. In the present example the variable-squared method has been used in determining the sums of squares.

The grand total of the whole experiment being 16,309 and there being 390 plots in the whole series of experiments, the correction factor (c. f.) is equal to

$$\frac{(16309)^2}{390} = 682008.9256,$$

a sum which has to be deducted from all crude sums of squares to enable us to get the true sum of squares for each particular item.

The total sum of squares for the whole experiment as shown in the last column of Table XLVI is the total of these figures, 734690 minus 682008.9256 (c. f.) or 52681.0744.

TABLE XLVI

*Yields of Pusa oats in lbs. from plots 1,000 square feet in area*

Locality	Seasons	Blocks	VARIETIES												Block Totals	Total sums of squares	
			A	B	C	D	E	F	G	H	I	J	K	L			M
Pusa . . .	1931-32	1	26.5	35.0	29.5	39.5	43.0	37.0	30.5	28.5	36.5	26.5	51.0	43.0	466.0	59,445.50	
		2	38.0	28.5	24.0	31.5	44.5	33.0	18.5	26.0	38.5	40.0	59.0	28.5	434.0		
		3	22.5	23.0	23.5	26.5	26.0	22.0	22.0	27.0	30.5	24.0	39.0	29.5	336.5		
		4	18.0	17.5	19.5	22.0	27.0	28.0	31.5	18.5	19.5	25.0	23.0	34.0	32.0		315.5
		5	22.5	19.5	25.5	25.0	30.0	32.5	27.5	21.5	15.5	28.5	22.5	38.5	28.0		337.0
		TOTALS	127.5	123.5	122.0	131.5	154.5	174.0	151.0	111.0	116.5	159.0	136.0	221.5	161.0		1,889.0
	1932-33	1	37.5	25.0	61.0	35.0	44.5	48.5	53.0	41.0	54.5	61.0	36.5	59.0	615.5	150,618.76	
		2	31.5	27.5	55.5	36.0	47.0	48.5	55.5	27.5	47.0	56.5	41.0	53.5	59.0		586.0
		3	28.0	30.5	61.0	46.0	37.0	58.0	57.0	32.5	51.0	58.5	28.0	51.0	54.5		593.0
		4	32.5	34.5	60.0	51.0	50.5	47.5	55.0	37.5	62.5	56.5	33.0	55.5	54.0		630.0
		5	40.5	29.5	58.0	57.5	52.5	49.0	51.5	31.5	53.5	51.0	47.5	42.5	59.5		624.0
		TOTALS	170.0	147.0	295.5	225.5	231.5	251.5	272.0	170.0	268.5	283.5	186.0	261.5	286.0		3,048.5
	1933-34	1	37.5	30.5	68.0	41.0	47.0	43.0	52.5	42.5	44.5	49.5	42.5	61.5	66.0	626.0	188,579.75
		2	48.0	35.5	71.0	52.0	57.0	48.5	55.5	51.5	55.5	55.5	46.5	57.5	57.5	691.5	
		3	49.0	38.0	67.5	52.5	57.5	48.5	59.0	48.0	52.0	51.5	48.5	55.0	63.5	690.5	
		4	54.0	38.5	68.5	54.5	60.0	49.0	57.0	52.5	54.0	56.5	49.0	69.0	70.0	732.5	
		5	48.0	32.0	64.5	49.5	49.0	38.0	56.5	48.0	48.0	67.5	42.5	54.5	62.0	660.5	
		TOTALS	236.5	174.5	339.5	249.5	270.5	227.0	280.5	242.5	254.0	280.5	229.0	297.5	319.0	3,400.5	
VARIETAL TOTALS FOR PUSA .			534.0	445.0	757.0	606.5	656.5	652.5	703.5	523.5	639.0	723.0	551.0	780.5	766.0	8,338.0	

# PLANT BREEDING AND AGRICULTURAL PROBLEMS.

Karnal	1931-32	44.5	29.0	44.0	48.5	42.0	30.5	50.5	40.5	41.0	46.5	51.5	19.0	38.0	525.5
1		34.5	37.0	42.5	39.0	40.5	44.0	47.0	43.0	29.5	53.5	45.5	34.5	25.5	516.0
2		43.0	27.0	36.0	38.5	33.5	32.5	44.5	39.0	31.5	37.5	30.0	38.0	36.0	467.0
3		42.0	23.0	46.0	42.0	26.5	34.0	44.0	33.5	36.0	42.0	34.0	39.5	44.0	486.5
4		42.5	30.5	42.0	35.0	36.0	31.0	32.5	26.5	26.0	42.0	36.0	28.0	34.5	442.5
TOTALS		206.5	146.5	210.5	203.0	178.5	172.0	218.5	182.5	164.0	221.5	197.0	159.0	178.0	2,437.5
															94,786.25
1932-33		29.0	27.5	36.0	30.5	35.0	36.0	46.0	27.5	33.0	45.0	32.0	56.5	55.0	489.0
2		36.0	22.0	48.0	45.0	43.0	34.0	48.0	33.0	34.0	57.0	38.0	47.0	58.0	543.0
3		38.0	29.5	47.0	48.0	48.0	42.0	52.0	45.0	36.0	57.0	42.0	64.0	62.0	610.5
4		40.0	29.5	55.0	45.5	50.0	40.0	57.0	48.5	40.5	50.5	40.5	65.0	52.5	609.5
5		40.0	22.0	52.5	48.5	42.0	35.5	48.0	31.0	34.5	59.0	40.0	47.0	60.0	560.0
TOTALS		183.0	130.5	238.5	217.5	218.0	187.5	251.0	180.0	178.0	268.5	192.5	279.5	287.5	2,812.0
															128,555.00
1933-34		40.0	27.0	38.5	38.5	34.0	34.5	35.5	33.5	35.5	37.5	39.5	39.5	40.5	474.0
2		36.5	26.5	38.5	32.0	35.0	33.0	33.5	30.5	40.5	37.5	32.5	35.0	50.0	466.0
3		39.5	34.0	42.0	48.0	43.5	43.0	41.5	37.5	41.0	39.0	35.5	45.0	49.5	539.0
4		45.5	31.0	42.0	36.5	48.5	46.5	48.0	39.5	48.5	49.5	40.0	53.0	56.5	585.0
5		55.5	37.5	57.5	50.0	52.5	52.5	54.5	43.0	51.0	47.0	54.0	46.0	56.5	657.5
TOTALS		217.0	156.0	218.5	205.0	213.5	209.5	218.0	184.0	216.5	210.5	201.5	218.5	253.0	2,721.5
															117,704.75
VARIETAL TOTALS FOR KARNAL		606.5	433.0	667.5	625.5	610.0	569.0	687.5	546.5	558.5	700.5	591.0	657.0	718.5	7,971.0
COMBINED VARIETAL TOTALS FOR PUSA AND KARNAL.		1,140.5	878.0	1,424.5	1,232.0	1,266.5	1,221.5	1,391.0	1,070.0	1,197.5	1,423.5	1,142.0	1,437.5	1,484.5	16,309.0

Sum of squares due to varieties, ignoring the effects of all other variables, is the sum of squares of total yields of each variety divided by the number of plots\* contributing to each total, minus the correction factor

$$= \frac{(1140.5)^2 + (878.0)^2 + (1424.5)^2 + \dots + (1484.5)^2}{30} - \text{c.f.} = 12297.1744$$

(see bottom of Table XLIX).

Similarly sum of squares due to seasons

$$= \frac{(4326.5)^2 + (5860.5)^2 + (6122.0)^2}{130} - \text{c.f.} = 14475.2784$$

(see bottom of Table XLVIII).

Sum of squares due to localities

$$= \frac{(8338)^2 + (7971)^2}{195} - \text{c.f.} = 345.3564 \text{ (see bottom of Table XLVII)}$$

and finally the sum of squares due to blocks

$$= \frac{(3196.0)^2 + (3236.5)^2 + \dots + (3281.0)^2}{78} - \text{c.f.} = 197.7744$$

(also see bottom of Table XLVII).

Having determined the sums of squares for the individual items shown above, it remains for us to calculate the interactions between any two of these variables taken together and finally the interactions between any three of these items combined together. The sum of squares contributed by all these components deducted from the total sum of squares will ultimately yield the sum of squares for the residual error.

### INTERACTIONS

The combined effects of any two variables taken at a time enables us to study whether these have or have not influenced the yields more than would result from the simple additive effects of these two components. Thus in Table XLVII we see that the sum of squares due to the combined effects of blocks and localities is equal to 1278.3544, which is much greater than the additive effect of these two variables as represented by their sums of squares, *i.e.*,  $197.7744 + 345.3564$  or 543.1308. This suggests that the interaction of blocks and localities has exerted a definite influence on the variability of the experiment.

The interaction between any two variables such as blocks and localities is indicated by blocks  $\times$  localities and is calculated as follows :

Interaction between blocks  $\times$  localities = S. S. (blocks  $\times$  localities) - (S. S. blocks + S. S. localities), where S. S. represents the sum of squares of a particular item.

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\* In determining the sums of squares of any variable, the squares of individual totals are added together and always divided by  $n$  which is equal to the number of plots which make up the yields of such totals and from this, of course, the correction factor is deducted.

Interactions between two variables taken at a time are shown in Tables XLVII to LII.

TABLE XLVII

*Combined blocks  $\times$  localities*

Blocks	Localities		Block totals
	Pusa	Karnal	
1 . . . . .	1707.5	1488.5	3196.0
2 . . . . .	1711.5	1525.0	3236.5
3 . . . . .	1620.0	1616.5	3236.5
4 . . . . .	1678.0	1681.0	3359.0
5 . . . . .	1621.0	1660.0	3281.0
Locality totals . . . . .	8338.0	7971.0	16309.0

The block yields in each locality in the above table have been derived by adding together block yields of the three seasons in each locality. Thus the first block yield for Pusa, *viz.*, 1707.5 = 466.0 + 615.5 + 626.0 lbs.

Total sum of squares for blocks  $\times$  localities

$$= \frac{(1707.5)^2 + (1488.5)^2 + \dots + (1660.0)^2}{39} - \text{c.f.} = \frac{26648204.00}{39} - \text{c.f.} \\ = 1278.3544.$$

Sum of squares for blocks

$$= \frac{(3196.0)^2 + (3236.5)^2 + \dots + (3281.0)^2}{78} - \text{c.f.} = \frac{53212122.50}{78} - \text{c.f.} \\ = 197.7744.$$

Sum of squares for localities

$$= \frac{(8338.0)^2 + (7971.0)^2}{195} - \text{c.f.} = \frac{133059085.00}{195} - \text{c.f.} = 345.3564$$

$\therefore$  Interaction between blocks  $\times$  localities

$$= \text{S. S. blocks} \times \text{localities} - (\text{S. S. blocks} + \text{S. S. localities})$$

$$= 1278.3544 - (197.7744 + 345.3564) = 735.2236$$



Table XLVIII shows the combined effect of blocks  $\times$  seasons.

TABLE XLVIII

*Combined blocks  $\times$  seasons*

				Seasons			Block totals
				1931-32	1932-33	1933-34	
	.	.	.	991.5	1104.5	1100.0	3196.0
2	.	.	.	950.0	1129.0	1157.5	3236.5
3	.	.	.	803.5	1203.5	1229.5	3236.5
4	.	.	.	802.0	1239.5	1317.5	3359.0
5	.	.	.	779.5	1184.0	1317.5	3281.0
Seasonal totals .				4326.5	5860.5	6122.0	16309.0

The block yields per season in this table have been obtained by summing the block yields in the two localities for each season. Thus  $991.5 = 466.0 + 525.5$ .

Total sum of squares for blocks  $\times$  seasons

$$= \frac{(991.5)^2 + (1104.5)^2 + \dots + (1317.5)^2}{26} - \text{c.f.} = \frac{18196287.50}{26} - \text{c.f.}$$

$$= 17848.2844$$

Sum of squares for blocks as already determined = 197.7744

$$\text{Sum of squares for seasons} = \frac{(4326.5)^2 + (5860.5)^2 + (6122.0)^2}{130} - \text{c.f.}$$

$$= \frac{90542946.50}{130} - \text{c.f.} = 14475.2784$$

$\therefore$  Interaction between blocks  $\times$  seasons

$$= \text{S. S. blocks} \times \text{seasons} - (\text{S. S. blocks} + \text{S. S. seasons})$$

$$= 17848.2844 - (197.7744 + 14475.2784) = 3175.2316$$

The combined effect of blocks  $\times$  varieties is shown in Table XLIX.

TABLE XLIX  
*Combined blocks × varieties*

Blocks	VARIETIES.													Block totals
	A	B	C	D	E	F	G	H	I	J	K	L	M	
1 . . .	215.0	174.0	277.0	233.0	242.0	235.5	274.5	215.5	237.0	276.0	228.5	286.5	301.5	3196.0
2 . . .	224.5	177.0	279.5	228.0	254.0	252.5	277.5	204.0	232.5	298.5	243.5	286.5	278.5	3236.5
3 . . .	220.0	182.0	277.0	254.0	246.0	250.0	276.0	224.0	238.5	274.0	208.0	292.0	295.0	3236.5
4 . . .	232.0	174.0	291.0	251.5	262.5	245.0	292.5	225.0	261.0	280.0	219.5	316.0	309.0	3359.0
5 . . .	249.0	171.0	300.0	265.5	262.0	238.5	270.5	201.5	228.5	295.0	242.5	256.5	300.5	3281.0
Varietal totals .	1140.5	878.0	1424.5	1232.0	1266.5	1221.5	1391.0	1070.0	1197.5	1423.5	1142.0	1437.5	1484.5	16309.0

The block yields per variety in the above table have been obtained by summing together the plot yields of each variety, in each block, in both the localities, in all three seasons. Thus 215.0 in the first row and first column is equal to  $26.5 + 37.5 + 37.5 + 44.5 + 29.0 + 40.0$ .

Total sum of squares for blocks  $\times$  varieties

$$= \frac{(215.0)^2 + (174.0)^2 + \dots + (300.5)^2}{6} - \text{c.f.} = \frac{4173698.00}{6} - \text{c.f.} \\ = 13607.4077$$

Sum of squares for blocks = 197.7744

Sum of squares for varieties

$$= \frac{(1140.5)^2 + (878.0)^2 + \dots + (1484.5)^2}{30} - \text{c.f.} = \frac{20829183.00}{30} - \text{c.f.} \\ = 12297.1744$$

$\therefore$  Interaction between blocks  $\times$  varieties

= S. S. blocks  $\times$  varieties — (S. S. varieties + S. S. blocks)

=  $13607.4077 - (197.7744 + 12297.1744) = 1112.4589$

The combined effect of seasons  $\times$  localities is shown in the following table :—

TABLE L

*Combined seasons  $\times$  localities*

Localities	Seasons			Locality totals
	1931-32	1932-33	1933-34	
Pusa . . .	1889.0	3048.5	3400.5	8338.0
Karnal . . .	2437.5	2812.0	2721.5	7971.0
Seasonal totals . .	4326.5	5860.5	6122.0	16309.0

The seasonal yields in the table represent the total yields of all varieties per season in each locality.

Total sum of squares for seasons  $\times$  localities

$$= \frac{(1889.0)^2 + (3048.5)^2 + \dots + (2721.5)^2}{65} - \text{c.f.} = \frac{45680386.00}{65} - \text{c.f.} \\ = 20766.2444$$

Sums of squares for seasons and localities are 14475.2784 and 345.3564 respectively.

$\therefore$  Interaction between seasons and localities

= S. S. seasons  $\times$  localities — (S. S. seasons + S. S. localities)

=  $20766.2444 - (14475.2784 + 345.3564) = 5945.6096$

In a like manner the combined effects of varieties  $\times$  seasons are tabulated on page 133.

TABLE LI  
Combined varieties  $\times$  seasons

Seasons	VARIETIES													Seasonal totals
	A	B	C	D	E	F	G	H	I	J	K	L	M	
1931-32 . . .	334.0	270.0	332.5	334.5	333.0	346.0	369.5	293.5	280.5	380.5	333.0	380.5	339.0	4326.5
1932-33 . . .	353.0	277.5	534.0	443.0	449.5	439.0	523.0	350.0	446.5	552.0	378.5	541.0	573.5	5860.5
1933-34 . . .	453.5	330.5	558.0	454.5	484.0	436.5	498.5	426.5	470.5	491.0	480.5	516.0	572.0	6122.0
Varietal totals . . .	1140.5	878.0	1424.5	1232.0	1266.5	1221.5	1391.0	1070.0	1197.5	1423.5	1142.0	1437.5	1484.5	16309.0

The yields per variety in this table have been obtained by combining together the total yields of each variety secured from both the localities per season.

Total sum of squares for varieties  $\times$  seasons

$$= \frac{(334.0)^2 + (270.0)^2 + \dots + (572.0)^2}{10} - \text{c.f.} = \frac{7126204.00}{10} - \text{c.f.} = 30611.4744$$

Sums of squares for varieties and seasons

$$= 12297.1744 \text{ and } 14475.2784 \text{ respectively.}$$

$$\begin{aligned} &\therefore \text{Interaction between varieties} \times \text{seasons} \\ &= \text{S. S. varieties} \times \text{seasons} - (\text{S. S. varieties} + \text{S. S. seasons}) \\ &= 30611.4744 - (12297.1744 + 14475.2784) = 3839.0216. \end{aligned}$$

Finally, the effect of combined varieties  $\times$  localities is brought out in Table LII shown on page 135.

The varietal yields represented in the above table have been obtained by summing together all the plot yields, under each variety in the three seasons, under consideration at Pusa and Karnal respectively.

Total sum of squares for varieties  $\times$  localities

$$= \frac{(534.0)^2 + (445.0)^2 + \dots + (718.5)^2}{15} - \text{c.f.} = \frac{10439484.50}{15} - \text{c.f.} = 13956.7077.$$

Sums of squares for varieties and localities are 12297.1744 and 345.3564 respectively.

$$\begin{aligned} &\therefore \text{Interaction between varieties} \times \text{localities} \\ &= \text{S. S. varieties} \times \text{localities} - (\text{S. S. varieties} + \text{S. S. localities}) \\ &= 13956.7077 - (12297.1744 + 345.3564) = 1314.1769. \end{aligned}$$

Interactions between the undermentioned variables have thus been determined :—

- (1) Blocks  $\times$  localities
- (2) Blocks  $\times$  seasons
- (3) Blocks  $\times$  varieties
- (4) Seasons  $\times$  localities
- (5) Varieties  $\times$  seasons
- and (6) Varieties  $\times$  localities.

Now the second order interactions, or the interactions between three variables taken at a time, are to be calculated. They are interactions between :—

- (1) Localities  $\times$  seasons  $\times$  varieties
- (2) Localities  $\times$  seasons  $\times$  blocks
- (3) Localities  $\times$  blocks  $\times$  varieties
- and (4) Seasons  $\times$  blocks  $\times$  varieties.

Table LIII shows the combined effect of localities  $\times$  seasons  $\times$  varieties,

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TABLE LII  
*Combined varieties × localities*

Localities	VARIETIES													Locality totals
	A	B	C	D	E	F	G	H	I	J	K	L	M	
Pusa . . . . .	534.0	445.0	757.0	606.5	656.5	652.5	703.5	523.5	639.0	723.0	551.0	780.5	766.0	8338.0
Karnal . . . . .	606.5	433.0	667.5	625.5	610.0	569.0	687.5	546.5	558.5	700.5	591.0	657.0	718.5	7971.0
Varietal totals . . . . .	1140.5	878.0	1424.5	1232.0	1266.5	1221.5	1391.0	1070.0	1197.5	1423.5	1142.0	1437.5	1484.5	16309.0

TABLE LIII

*Combined localities × seasons × varieties (excluding block effect)*

Localities	Seasons	VARIETIES												Ses- sonal totals	Locality totals	
		A	B	C	D	E	F	G	H	I	J	K	L			M
Pusa	1931-32	127.5	123.5	122.0	131.5	154.5	174.0	151.0	111.0	116.5	159.0	136.0	221.5	161.0	1889.0	8338.0
	1932-33	170.0	147.0	295.5	225.5	231.5	251.5	272.0	170.0	268.5	283.5	186.0	261.5	286.0	3048.5	
	1933-34	236.5	174.5	339.5	249.5	270.5	227.0	280.5	242.5	254.5	280.5	229.0	297.5	319.0	3400.5	
Karnal	1931-32	206.5	146.5	210.5	203.0	178.5	172.0	218.5	182.5	164.0	221.5	197.0	159.0	178.0	2437.5	7971.0
	1932-33	183.0	130.5	238.5	217.5	218.0	187.5	251.0	180.0	178.0	268.5	192.5	279.5	287.5	2812.0	
	1933-34	217.0	156.0	218.5	205.0	213.5	209.5	218.0	184.0	216.5	210.5	201.5	218.5	253.0	2721.5	
Varietal totals		1140.5	878.0	1424.5	1232.0	1266.5	1221.5	1391.0	1070.0	1197.5	1423.5	1142.0	1437.5	1484.5	16309.0	

The varietal totals in this table have been secured by summing together the plot yields of each variety in all the five blocks in each experiment.

Total sum of squares for localities  $\times$  seasons  $\times$  varieties

$$= \frac{(127.5)^2 + (123.5)^2 + \dots + (253.0)^2}{5} - \text{c.f.} = \frac{3616745.00}{5} - \text{c.f.}$$

$$= 41340.0744$$

Sums of squares for localities, seasons and varieties are 345.3564, 14475.2784 and 1297.1744 respectively.

Interactions between seasons  $\times$  localities, varieties  $\times$  seasons and varieties  $\times$  localities have also been calculated in previous tables and are 5955.6086, 3839.0216 and 1314.1769 respectively.

$$\therefore \text{Interaction between localities} \times \text{seasons} \times \text{varieties}$$

$$= \text{S. S. localities} \times \text{seasons} \times \text{varieties} - (\text{S. S. localities} + \text{S. S. seasons} + \text{S. S. varieties} + \text{interactions between seasons} \times \text{localities} + \text{varieties} \times \text{seasons} + \text{varieties} \times \text{localities})$$

$$= 41340.0744 - (345.3564 + 14475.2784 + 12297.1744 + 5945.6096 + 3839.0216 + 1314.1769) = 3123.4571.$$

In Table LIV, the combined effects of localities  $\times$  seasons  $\times$  blocks are demonstrated.

TABLE LIV

*Combined localities  $\times$  seasons  $\times$  blocks (excluding varietal effect)*

Localities	Seasons	BLOCKS					Seasonal totals	Locality totals
		1	2	3	4	5		
Pusa . . . . .	1931-32 .	466.0	434.0	336.5	315.5	337.0	1889.0	8338.0
	1932-33 .	615.5	586.0	593.0	630.0	624.0	3048.5	
	1933-34 .	626.0	691.5	690.5	732.5	660.0	3400.5	
Karnal . . . . .	1931-32 .	525.5	516.0	467.0	486.5	442.5	2437.5	7971.0
	1932-33 .	489.0	543.0	610.5	609.5	560.0	2812.0	
	1933-34 .	474.0	466.0	539.0	585.0	657.5	2721.5	
Block totals . . . . .		3196.0	3236.5	3236.5	3359.0	3281.0	16309.0	16309.0



The block yields in this table have been obtained by summing the plot yields of all the varieties in each season and in each locality.

Total sum of squares for localities  $\times$  seasons  $\times$  blocks

$$= \frac{(466.0)^2 + (434.0)^2 + \dots + (657.5)^2}{13} - \text{c.f.} = \frac{9202648}{13} - \text{c.f.} \\ = 25887.0744.$$

Sums of squares for localities, seasons and blocks are 345.3564, 14475.2784 and 197.7744 respectively.

Interactions between blocks  $\times$  localities, seasons  $\times$  localities and blocks  $\times$  seasons are equal to 735.2236, 5945.6096 and 3175.2316 respectively.

$$\therefore \text{Interaction between localities} \times \text{seasons} \times \text{blocks} \\ = \text{S. S. localities} \times \text{seasons} \times \text{blocks} - (\text{S. S. localities} + \text{S. S. seasons} + \\ \text{S. S. blocks} + \text{interactions between blocks} \times \text{localities} + \text{seasons} \times \\ \text{localities} + \text{blocks} \times \text{seasons}) \\ = 25887.0744 - (345.3564 + 14475.2784 + 197.7744 + 735.2236 + 5945.6096 \\ + 3175.2316) = 1012.6004.$$

The combined effect of localities  $\times$  blocks  $\times$  varieties is represented in Table LV given on page 139.

The varietal yields in this table have been obtained by combining the plot yields of each variety in the three seasons for each locality. Thus  $101.5 = 26.5 + 37.5 + 37.5$ .

Total sum of squares for localities  $\times$  blocks  $\times$  varieties

$$= \frac{(101.5)^2 + (90.5)^2 + \dots + (151.1)^2}{3} - \text{c.f.} = \frac{2096725.00}{3} - \text{c.f.} \\ = 16899.4077.$$

Sums of squares for localities, blocks and varieties are 345.3564, 197.7744 and 12297.1744 respectively.

Again interactions between blocks  $\times$  localities, blocks  $\times$  varieties and varieties  $\times$  localities are 735.2236, 1112.4589 and 1314.1769 respectively.

$$\therefore \text{Interaction between localities} \times \text{blocks} \times \text{varieties} \\ = \text{S. S. localities} \times \text{blocks} \times \text{varieties} - (\text{S. S. localities} + \text{S. S. blocks} + \\ \text{S. S. varieties} + \text{interactions between blocks} \times \text{localities} + \text{blocks} \times \\ \text{varieties} + \text{varieties} \times \text{localities}) \\ = 16899.4077 - (345.3564 + 197.7744 + 12297.1744 + 735.2236 + \\ 1112.4589 + 1314.1769) = 897.2451$$

At last, the interaction between seasons  $\times$  blocks  $\times$  varieties is tabulated (Table LVI).

TABLE LV  
*Combined localities × blocks × varieties (excluding seasonal effect)*

Localities	Blocks	VARIETIES													Block totals	Locality totals
		A	B	C	D	E	F	G	H	I	J	K	L	M		
Pusa	1	101.5	90.5	153.5	115.5	131.0	134.5	142.5	114.0	127.5	147.0	105.5	171.5	168.0	1707.5	8338.0
	2	117.5	91.5	150.5	112.0	135.5	141.5	144.0	97.5	128.5	150.5	127.5	170.0	145.0	1711.5	
	3	99.5	91.5	152.0	119.5	121.0	132.5	138.0	102.5	130.0	140.5	100.5	145.0	147.5	1620.0	
	4	104.5	90.5	148.0	127.5	137.5	124.5	143.5	108.5	136.0	138.0	105.0	158.5	156.0	1678.0	
	5	111.0	81.0	148.0	132.0	131.5	119.5	135.5	101.0	117.0	147.0	112.5	135.5	149.5	1621.0	
Karnal	1	113.5	83.5	118.5	117.5	111.0	101.0	132.0	101.5	109.5	129.0	123.0	115.0	133.5	1438.5	7971.0
	2	107.0	85.5	129.0	116.0	118.5	111.0	133.5	106.5	104.0	148.0	116.0	116.5	133.5	1525.0	
	3	120.5	90.5	125.0	134.5	125.0	117.5	138.0	121.5	108.5	133.5	107.5	147.0	147.5	1616.5	
	4	127.5	83.5	143.0	124.0	125.0	120.5	149.0	116.5	125.0	142.0	114.5	157.5	153.0	1631.0	
	5	138.0	90.0	152.0	133.5	130.5	119.0	135.0	100.5	111.5	148.0	130.0	121.0	151.1	1660.0	
Varietal totals	..	1140.5	878.0	1424.5	1232.0	1266.5	1221.5	1391.0	1070.0	1197.5	1423.5	1142.0	1437.5	1484.5	16309.0	16309.0

TABLE LVI

*Combined seasons × blocks × varieties (excluding locality effects)*

Seasons	Blocks	VARIETIES													Block totals	Seasonal totals
		A	B	C	D	E	F	G	H	I	J	K	L	M		
1931-32	1	71.0	64.0	73.5	88.0	81.5	73.5	87.5	71.0	69.5	83.0	78.0	70.0	81.0	991.5	4326.5
	2	72.5	65.5	66.5	63.0	72.0	88.5	80.0	61.5	55.5	92.0	85.5	93.5	54.0	950.0	
	3	65.5	50.0	59.5	59.5	60.0	58.5	66.5	61.0	58.5	68.0	54.0	77.0	65.5	803.5	
	4	60.0	40.5	65.5	64.0	53.5	62.0	75.5	52.0	55.5	67.0	57.0	73.5	76.0	802.0	
	5	65.0	50.0	67.5	60.0	66.0	63.5	60.0	48.0	41.5	70.5	58.5	66.5	62.5	779.5	
1932-33	1	66.5	52.5	97.0	65.5	79.5	84.5	39.0	68.5	87.5	106.0	68.5	115.5	114.0	1104.5	5860.5
	2	67.5	49.5	103.5	81.0	90.0	82.5	103.5	60.5	81.0	113.5	79.0	100.5	117.0	1129.0	
	3	66.0	60.0	108.0	94.0	85.0	100.0	109.0	77.5	87.0	115.5	70.0	115.0	116.5	1203.5	
	4	72.5	64.0	115.0	96.5	100.5	87.5	112.0	81.0	103.0	107.0	73.5	120.5	106.5	1239.5	
	5	80.5	51.5	110.5	106.0	94.5	84.5	99.5	62.5	88.0	110.0	87.5	89.5	119.5	1184.0	
1933-34	1	77.5	57.5	106.5	79.5	81.0	77.5	88.0	76.0	80.0	87.0	82.0	101.0	106.5	1100.0	6122.0
	2	84.5	62.0	109.5	84.0	92.0	81.5	94.0	82.0	96.0	93.0	79.0	92.5	107.5	1157.5	
	3	88.5	72.0	109.5	100.5	101.0	91.5	100.5	85.5	93.0	90.5	84.0	100.0	113.0	1229.5	
	4	99.5	69.5	110.5	91.0	108.5	95.5	105.0	92.0	102.5	106.0	89.0	122.0	126.5	1317.5	
	5	103.5	69.5	122.0	99.5	101.5	90.5	111.0	91.0	99.0	114.5	96.5	100.5	118.5	1317.5	
Varietal totals	..	1140.5	878.0	1424.5	1232.0	1266.5	1221.5	1391.0	1070.0	1197.5	1423.5	1142.0	1437.5	1484.5	16309.0	16309.0

The varietal totals have been obtained in the above table by summing together the plot yields from the two localities per block per season.

Total sum of squares for seasons  $\times$  blocks  $\times$  varieties

$$= \frac{(71.0)^2 + (64.0)^2 + \dots + (118.5)^2}{2} - \text{c.f.} = \frac{1438694.00}{2} - \text{c.f.} \\ = 37338.0744$$

Sums of squares for seasons, blocks and varieties are 14475.2784, 197.7744 and 12297.1744 respectively.

Interactions between blocks  $\times$  seasons, blocks  $\times$  varieties and varieties  $\times$  seasons are respectively 3175.1216, 1112.4589 and 3839.0216.

$\therefore$  Interaction between seasons  $\times$  blocks  $\times$  varieties

$$= \text{S. S. seasons} \times \text{blocks} \times \text{varieties} - (\text{S. S. seasons} + \text{S. S. blocks} + \text{S. S. varieties} + \text{interactions between blocks} \times \text{seasons} + \text{blocks} \times \text{varieties} + \text{varieties} \times \text{seasons})$$

$$= 37338.0744 - (14475.2784 + 197.7744 + 12297.1744 + 3175.2316 + 1112.4589 + 3839.0216) = 2241.1351$$

Sums of squares due to the residual error is equal to the difference between the total sum of squares for the experiment and the total of all the other sums of squares.

*Degrees of freedom.*—Altogether there are 390 plots in the whole experiment and hence the total degrees of freedom are 390 — 1 or 389. These could be divided further as shown in column 2 of Table LVII.

The analysis of variance is given in Table LVII.

TABLE LVII  
*Analysis of variance*

Due to	Degrees of freedom	Sums of squares	Mean squares	MAHA
1	2	3	4	Observed
1. Blocks . . . . .	4	197.7744	49.4436	2.410
2. Localities . . . . .	1	345.3564	345.3564	16.835
3. Seasons . . . . .	2	14475.2784	7237.6392	352.816
4. Varieties . . . . .	12	12297.1744	1024.7645	49.954
Interactions.				8.960
5. Blocks $\times$ localities . . . . .	4	735.2236	183.8059	19.348
6. Blocks $\times$ seasons . . . . .	8	3175.2316	396.9039	1.129
7. Blocks $\times$ varieties . . . . .	48	1112.4589	23.1762	144.916
8. Seasons $\times$ localities . . . . .	2	5945.6096	2972.8048	7.797
9. Varieties $\times$ seasons . . . . .	24	3839.0216	159.9592	5.338
10. Varieties $\times$ localities . . . . .	12	1314.1769	109.5147	6.344
11. Localities $\times$ seasons $\times$ varieties . . . . .	24	3123.4571	130.1439	1.791
12. Localities $\times$ seasons $\times$ blocks . . . . .	8	1062.6024	132.8253	2.511
13. Localities $\times$ blocks $\times$ varieties . . . . .	48	897.2451	18.6926	4.605
14. Seasons $\times$ blocks $\times$ varieties . . . . .	96	2241.1351	23.3452	1.000
15. Residual error . . . . .	96	1944.3900	20.139	..
TOTAL . . . . .	389	52681.0744	135.4269	1.1380

*add.* But since the several blocks in different seasons and localities do not correspond, the block sum of squares is the sum of items 1, 5, 6 and 12, giving 24 degrees of freedom for 'blocks', similarly residual sum of squares for error will be the sum of items 7, 13, 14 and 15. This will obviously alter the calculations in columns 4, 5 and 6 and the conclusions drawn therefrom in the succeeding pages.

The mean squares in this table have been calculated for each item by dividing the respective sums of squares by their appropriate degrees of freedom. The

statistical significance of each mean square is determined by Mahalanobis'  $x$ -test by taking the ratio of each mean square to the mean square for residual error. This observed ratio  $\frac{V_1}{V_2}$  is shown in column 5 and the expected critical ratios from Mahalanobis' tables for the  $P = 0.01$  level are placed against each in column 6.

It will be noted that the mean square for blocks is not significant at the one per cent level but is significant at the lower level,  $P = 0.05$ . The mean square for the interaction, localities  $\times$  blocks  $\times$  varieties, is not significant, while all the other variances are definitely significant. This shows that we have, by eliminating the effects of different items, definitely brought about an improvement in the precision of the experiment. The mean square for error without any eliminations (total error) is 135.4269 whereas the residual error after elimination of other effects is only 20.6178, so that the improvement in precision obtained by using this method of analysis is  $\frac{135.4269}{20.6178} = 6.57$  times.

Table LVIII shows the improvement in precision obtained by reducing the experimental error of the whole experiment by eliminating various items from this. The mean square for error, without any eliminations of such effects as those brought about by blocks, seasons, etc., is 135.4269. It is reduced to 68.5552 if effects due to blocks, localities, seasons and varieties are excluded. If a further elimination of all interactions of the first order or those between any two variables taken at a time, is also made, the mean square error comes down to 33.9477. But if the interactions of the second order, or those between three variables at a time are also taken into account in addition to the above, as has been done in the analysis of variance shown in Table LVII, the variance for error is finally reduced to 20.6178. The improvement in precision in each case is shown in the last column of Table LVIII.

TABLE LVIII

*Comparison of experimental errors after the elimination of different effects*

Errors	Degrees of freedom	Sums of squares	Mean squares	Ratio of mean square error (1) to other mean squares	Improvement in precision
(1) Without elimination of any effects such as those due to blocks, etc.	389	52681.0744	135.4269	..	..
(2) After eliminating effects due to blocks, localities, seasons, and varieties.	370	25365.4908	68.5552	$\frac{135.4269}{68.5552}$	1.98 times.
(3) After eliminating effects due to (2) and also all interactions taken two at a time.	272	9233.7696	33.9477	$\frac{135.4269}{33.9477}$	3.98 times.
(4) After eliminating effects due to (3) and also all interactions taken three at a time.	96	1979.3099	20.6178	$\frac{135.4269}{20.6178}$	6.57 times.

Having established that there are significant differences in the yielding power of the oats in—

- (1) the two localities
  - (2) the three seasons
- and (3) the thirteen varieties under trial,

we have now to draw up tables of mean differences for these variables and compare the possible pairs of differences with the help of critical differences for the respective factors under consideration.

The residual variance is 20.6178. Therefore the standard error of the difference for each comparison is obtained by the square root of this number divided by  $n$ , the number of plots contributing to each total, and multiplying this quantity by  $\sqrt{2}$ .

The critical difference, as usual, is obtained by multiplying the standard error of the difference by the value of ' $t$ ' given in Fisher's tables for the 0.01 or 0.05 levels of significance.

Table LIX shows that the mean yield per plot, per variety, in the two localities, Pusa and Karnal, are 42.76 and 40.88 pounds with a mean difference of 1.88 lbs. The critical difference at the 1 per cent level being only 1.1985 lbs., the observed difference of 1.88 lbs. is statistically significant and suggests that the oat varieties generally yielded higher at Pusa than at Karnal.

TABLE LIX

*Mean yield per plot per variety at Pusa and Karnal*

Localities	Pusa	Karnal	S. E. of difference
Pusa . . .	..	-1.88	$\sqrt{\frac{20.6178 \times 2}{195}} = 0.46$ Critical difference at 1% level = 1.1985 lbs.
Karnal . . .	+1.88	..	
Mean . . .	42.76	40.88	

Table LX shows the differences of mean yields per plot per season and shows that significantly higher yields were obtained in 1932-33 than in 1931-32 and that the yields produced in 1933-34 were significantly higher than those secured in the previous two years.

TABLE LX

*Differences of mean yields per plot per season,  
ignoring the localities*

Seasons	1931-32	1932-33	1933-34
1931-32 . . .	..	+11.80	+13.81
1932-33 . . .	-11.80	..	+2.01
1933-34 . . .	-13.81	-2.01	..
Means . . .	33.28	45.08	47.09

$$\text{S. E. of difference} = \sqrt{\frac{20.6178 \times 2}{130}}$$

Critical difference at 0.01 level = 1.4507.

All the differences in the above table are therefore significant at the 1 per cent level.

The most important comparison, however, and in fact the main aim of the whole test, was the determination of significant differences in the yielding powers of the thirteen varieties of oats under observation. Table LXI provides all the possible sets of differences between mean yields of the varieties, irrespective of seasons and localities. Differences which are greater than the critical differences for  $P = 0.01$  and which are, therefore, significant at this level are in bold figures, thus + **10.75**. Those which are significant only at  $P = 0.05$  level are in italic figures, thus + *3.00*. Differences which are not significant are shown in ordinary figures. It may be pointed out that only positive and not negative significant differences have been shown in bold or in italic figures for the sake of convenience.

The conclusions are obvious and a detailed recapitulation of the significant differences in the above table seems unnecessary.

The five highest yielders are shown as *M*, *L*, *C*, *J*, and *G* which are ranked in this order but varieties *L*, *C*, *J*, and *G* are not statistically different from each other and may all be classed as yielders of the same order. *M* is statistically superior in yielding power to *G* only and not to any other type of these oats. We can safely conclude perhaps that in *C*, *J* and *G* we have three hybrids in which the high yielding capacity of the Pusa parents has been combined with plump grain and other good qualities of the Scotch Potato oats. This is a finding which should prove of immense use in arranging seed distribution programmes.

Hybrids *A*, *B*, *H* and *K*, on the other hand, have not done well. Although hybrids *A* and *B* had shown great promise in the preliminary cultural stages and were specially attractive for their very plump grains, their behaviour in the serial experiments indicates that these two types must not be distributed to any extent.

Tables LXII and LXIII furnish evidence of the behaviour of these thirteen oats under Pusa and Karnal conditions respectively. It is interesting to note how some types have changed places as regards rank and how the early maturing type *L* (B. S. 1) has yielded higher at Pusa than *M* (B. S. 2) which is about a couple of weeks later in maturity, whereas at Karnal these two oats have yielded in the reverse order.

TABLE LXI

*Differences of mean yields of different varieties, irrespective of seasons and localities*

Varieties	A	B	C	D	E	F	G	H	I	J	K	L	M
A	..	-10.75	+9.46	+3.05	+4.20	+2.70	+8.35	-2.35	+1.90	+9.43	+0.05	+9.90	+11.46
B	+10.75	..	+18.21	+11.80	+12.95	+11.45	+17.10	+6.40	+10.65	+18.18	+8.80	+18.65	+20.21
C	-9.46	-18.21	..	-6.41	-5.26	-6.76	-1.11	-11.81	-7.56	-0.03	-9.41	+0.44	+2.00
D	-3.05	-11.80	+6.41	..	+1.15	-0.35	+5.30	-5.40	-1.15	+6.38	-3.00	+6.85	+8.41
E	-4.20	-12.95	+5.26	-1.15	..	-1.50	+4.15	-6.55	-2.30	+5.23	-4.15	+5.70	+7.26
F	-2.70	-11.45	+6.76	+0.35	+1.50	..	+5.65	-5.05	-0.80	+6.73	-2.65	+7.20	+8.76
G	-8.35	-17.10	+1.11	-5.30	-4.15	-5.65	..	-10.70	-6.45	+1.08	-8.30	+1.55	+3.11
H	+2.35	-6.40	+11.81	+5.40	+6.55	+5.05	+10.70	..	+4.25	+11.78	+2.40	+12.25	+13.81
I	-1.90	-10.65	+7.56	+1.15	+2.30	+0.80	+6.45	-4.25	..	+7.53	-1.85	+8.00	+9.56
J	-9.43	-18.18	+0.03	-6.38	-5.23	-6.73	-1.08	-11.78	-7.53	..	-0.38	+0.47	+2.03
K	-0.05	-8.80	+9.41	+3.00	+4.15	+2.65	+8.30	-2.40	+1.85	+9.38	..	+9.85	+11.41
L	-9.90	-18.65	-0.44	-6.85	-5.70	-7.20	-1.55	-12.25	-8.00	-0.47	-9.85	..	+1.56
M	-11.46	-20.21	-2.00	-8.41	-7.26	-8.76	-3.11	-13.81	-9.56	-2.03	-11.41	-1.56	..
Mean yields in. lb.	38.02	29.27	47.48	41.07	42.22	40.72	46.37	35.67	39.92	47.45	38.07	47.92	49.48
Rank	11	13	3	7	6	8	5	12	9	4	10	2	1

S. E. of difference =  $\sqrt{\frac{20.6178 \times 2}{80}}$  Critical difference at the 1% level = 3.0188 Critical difference at the 5% level = 2.2971



TABLE LXII

*Differences of mean yields of different varieties at Pusa, irrespective of seasons*

Varieties	A	B	C	D	E	F	G	H	I	J	K	L	M
A	..	-5.93	+14.87	+4.83	+8.17	+7.90	+11.30	-0.70	+7.00	+12.60	+10.70	+16.43	+15.47
B	+5.93	..	+20.80	+10.76	+14.10	+13.83	+17.23	+5.23	+12.93	+18.53	+7.00	+22.36	+21.40
C	-14.87	-20.80	..	+10.04	-6.70	-6.97	-3.57	-15.57	-7.87	-2.27	-13.80	+1.56	+0.60
D	-4.83	-10.76	+10.04	..	+3.34	+3.07	+6.47	-5.53	+2.17	+7.77	-3.76	+11.60	+10.64
E	-8.17	-14.10	+6.70	-3.34	..	-0.27	-3.13	-8.87	-1.17	+4.43	-7.10	+8.26	+7.30
F	-7.90	-13.83	+6.97	-3.07	+0.27	..	+3.40	-8.60	-0.90	+4.70	-6.83	+8.53	+7.57
G	-11.30	-17.23	+3.57	-6.47	+3.13	-3.40	..	-12.00	-4.30	+1.30	-10.23	+5.13	+4.17
H	+0.70	-5.23	+15.57	+5.53	+8.87	+8.60	+12.00	..	+7.70	+13.30	+1.77	+17.13	+16.17
I	-7.00	-12.93	+7.87	-2.17	+1.17	+0.90	+4.30	-7.70	..	+5.60	-5.93	+9.43	+8.47
J	-12.60	-18.53	+2.27	-7.77	-4.43	-4.70	-1.30	-13.80	-5.60	..	-11.53	+3.83	+2.87
K	-10.70	-7.00	+13.80	+3.76	+7.10	+6.83	+10.23	-1.77	+5.93	+11.53	..	+15.36	+14.40
L	-16.43	-22.36	-1.56	-11.60	-8.26	+8.53	-5.13	-17.13	-9.43	-3.83	-15.36	..	-0.96
M	-15.47	-21.40	-0.60	-10.64	-7.30	-7.57	-4.17	-16.17	-8.47	-2.87	-14.40	+0.96	..
Mean yields in lb.	35.60	29.67	50.47	40.43	43.77	43.50	46.90	34.90	42.60	48.20	36.67	52.03	51.07
Rank	11	13	3	9	7	6	5	12	8	4	10	1	2

S. E. of mean difference =  $\sqrt{\frac{20.6178}{15} \times 2} = 1.6580$  Critical difference at the 1% level = 4.2707 Critical difference at the 5% level = 3.8571

TABLE LXIII  
Differences of mean yields of different varieties at Karnal, irrespective of seasons

Varieties	A	B	C	D	E	F	G	H	I	J	K	L	M
A	..	-11.55	+4.07	+1.27	+0.24	-2.50	+5.40	-4.00	-3.20	+6.27	-1.03	+3.37	+7.47
B	+11.55	..	+15.62	+12.82	+11.79	+9.65	+16.95	+7.55	+8.35	+17.82	+10.52	+14.92	+19.02
C	-4.07	-15.62	..	-2.80	-3.83	-6.57	-1.33	-8.07	+7.27	-2.20	-5.10	-0.70	-3.40
D	-1.27	-12.82	-2.80	..	-1.03	-3.77	+4.13	-5.27	-4.47	+5.00	-2.30	+2.10	+6.20
E	-0.24	-11.79	+3.83	+1.03	..	-2.74	+5.16	-4.24	-3.44	+6.03	-1.27	+3.13	+7.23
F	+2.50	-9.05	-6.57	+3.77	+2.74	..	+7.90	-1.50	-0.70	+8.77	+1.47	+5.87	+9.97
G	-5.40	-16.85	-1.33	-4.13	-5.16	-7.90	..	-9.40	-3.60	+0.87	-6.43	-2.03	+2.07
H	+4.00	-7.55	-8.07	+5.27	+4.24	+1.50	+9.40	..	+0.80	+10.27	+2.97	+7.37	+11.47
I	+3.20	-8.35	-7.27	+4.47	+3.44	+0.70	+8.60	-0.80	..	+9.47	+2.17	+6.57	+10.67
J	-6.27	-17.82	-2.20	-5.00	-6.03	-3.77	-0.87	-10.27	-9.47	..	-7.30	-2.90	+1.20
K	+1.03	-10.52	-5.10	+2.30	+1.27	-1.47	+6.43	-2.97	-2.17	+7.30	..	+4.40	+8.50
L	-3.37	-0.70	-0.70	-2.10	-3.13	-5.87	+2.03	-7.37	-6.57	+2.90	-4.40	..	+4.10
M	-7.47	-19.02	-3.40	-6.20	-7.23	-9.97	-2.07	-11.47	-10.67	-1.20	-8.50	-4.10	..
Mean yields in lbs.	40.43	28.58	44.50	41.70	40.67	37.93	45.83	36.43	37.23	46.70	39.40	43.80	47.90
Rank	8	13	4	6	7	10	3	12	11	2	9	5	1

S. E. of mean difference =  $\sqrt{\frac{20.6178}{15} \times 2} = 1.6580$  Critical difference at the 1% level = 4.2707 Critical difference at the 5% level = 3.8571

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## CHAPTER XI

## SOIL-HETEROGENEITY AND THE ANALYSIS OF COVARIANCE

In theory, field trials should be conducted under uniform conditions of soil and culture. This, however, is an unattainable ideal since an absolutely uniform piece of land hardly exists in nature and, therefore, methods must be adopted which lessen or eliminate the effects of soil-heterogeneity. Fields which from inspection appear satisfactorily uniform have been shown by Harris [1915] and others to be very heterogeneous in many cases, and the extent to which even a small field can show variations in fertility has been demonstrated by the results of uniformity trials conducted at Pusa. A field about one-fourth of an acre in area which had received uniform cultural and other treatments was sown in three consecutive years with Pusa barley, type 21, Pusa 52 wheat and Pusa lentils, type 11, respectively. At harvest a substantial border was removed from all sides and the remaining field was sub-divided into 390 ultimate plots, each 4 feet square (Table LXIV). The produce from these ultimate units was harvested and threshed separately. Combinations of  $2 \times 3$  such ultimate plots were made for the purpose of drawing contour maps of the yields, the field being considered as consisting of 65 such combination plots, 5 plots running from West to East by 13 plots running North to South as shown in Fig. 20. Assuming that the average yield of each plot is located at the centre of a plot, the points at which the yields were 10, 20, 30, 40, etc., per cent above or below the mean yield of the crop were marked on the field plan by interpolation. These points were joined and the contour maps shown in the figure given on next page were constructed.

It is evident that the yield varied greatly between different sub-plots in the same field and that this heterogeneity was systematic to a considerable extent is also apparent as the fertility contour lines run to a very pronounced degree more or less parallel to the direction of the columns running North to South. The contour lines show a good deal of similarity in all the three maps shown on next page. The differences present are due, of course, to the variability in the yielding power of the different crops under consideration and its relation to the mean yield of the crop under the conditions of the experiment.

Generally speaking, soil-heterogeneity may exist either as a gradual change of productivity from one side of the field to the other corresponding to the line of slope or the pathway of irrigation, etc., or as random patches of ground of higher or lower fertility. This 'patchiness' of a field is due to adjacent plots resembling

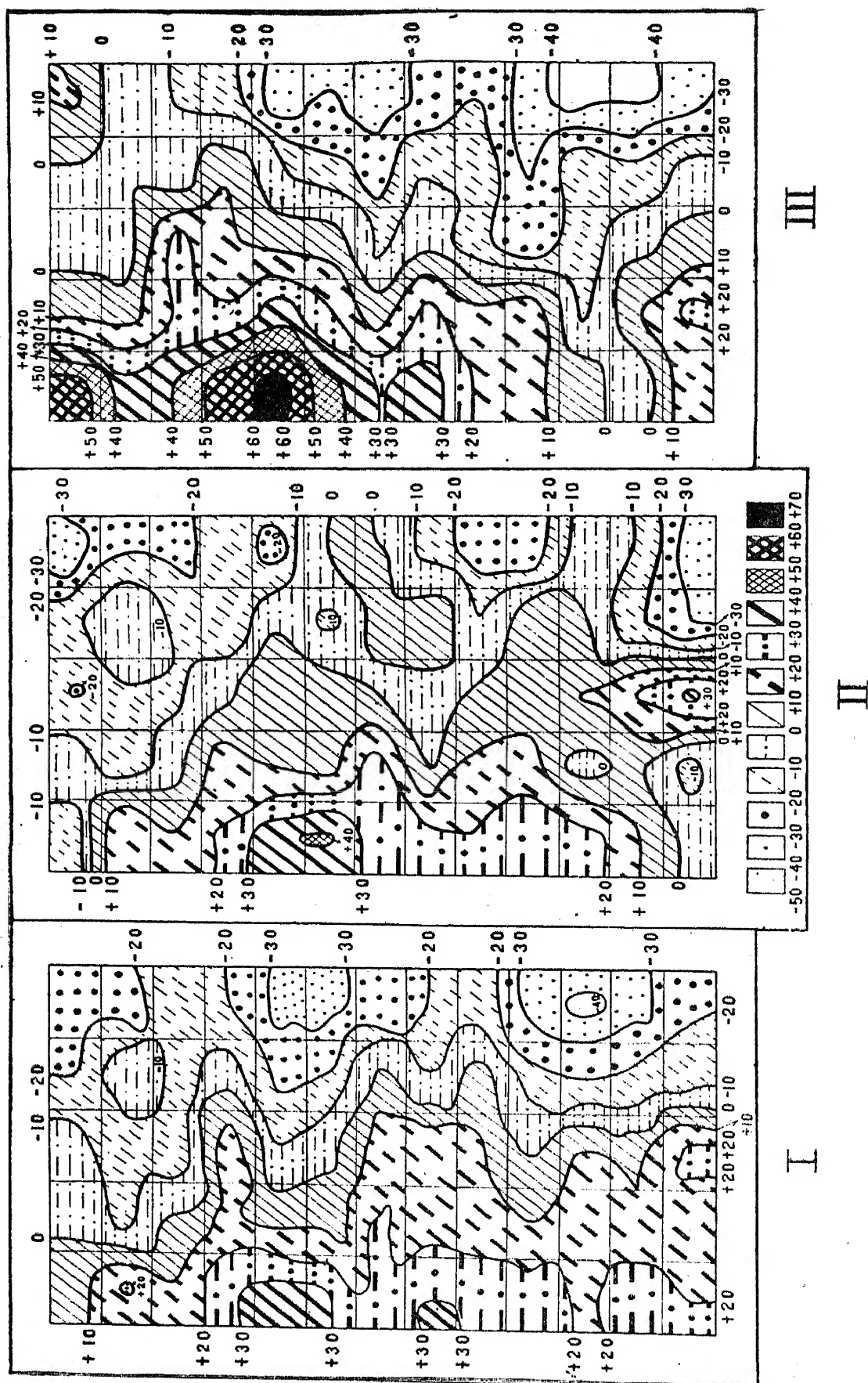


Fig. 20.—Contour maps of the yields of (i) barley, (ii) wheat and (iii) lentils obtained in three successive years from the same experimental field.

each other and represents the most common type of soil-heterogeneity. These irregularities in the field may sometimes be so powerful as to vitiate the results of varietal or breeding trials, giving significance to yields in situations where it is not actually present.

It must be remembered that the main object of the modern methods of field trials, and the application of refined methods of analysis to their results is to lessen, as far as possible, this effect of soil-heterogeneity. Although Fisher [1932] does not recommend in general the cropping of the experimental plots in the year previous to the experiment, as it involves double the labour of the experiment and a year's delay before the result is made available, it may be profitable if the experimenter has some measure of the nature of his field and knows in which direction the soil fertility varies. He has then the advantage of having valuable information to assist him in deciding on the lay-out of the experiment and on the correct size and shape of plots which he should choose. It is also possible for him then to discard fields of "patchy" fertility for comparative trials. In agricultural experimental and demonstration stations where the testing of improved varieties of crops or their response to different manurial or other treatment forms an important item of work, a knowledge of the heterogeneity of different fields proves very advantageous.

Harris [1915] has suggested that soil-heterogeneity may be measured by a coefficient which shows the degree of correlation between the yields of continuous plots. Fisher's analysis of variance may also be employed to determine the drift in the fertility of the field. Whereas, Harris' method provides a measure of heterogeneity present in the whole field, Fisher's analysis of variance method not only gives this measure but also clearly sets forth the direction of the fertility gradient and should, therefore, prove a more comprehensive method for such work.

For the purposes of this chapter, it will be sufficient, perhaps, to illustrate the calculations of the measure of soil-heterogeneity by these two methods in the experiment with wheat conducted in 1930-31 at Pusa.

#### (1) HARRIS' METHOD

The yields of ultimate plots of wheat are shown in Table LXIV. Two series of groupings hereafter called the  $1 \times 5$  combination and the  $2 \times 5$  combination, respectively, were made by totalling together the yields of one plot North to South and five plots East to West in the first case and two plots North to South and five plots East to West in the second case. Thus out of 390 ultimate plots, 78 combination plots could be made up for the  $1 \times 5$  combination and 39 combination plots formed for the  $2 \times 5$  combination. The yields of these two series of combination plots are shown in Tables LXV and LXVI respectively.

TABLE LXIV

*Yield of Pusa 52 wheat from ultimate plots 4 ft. × 4 ft. in area*

Row No.	YIELD IN GMS. FROM COLUMN NO.														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	70	220	265	230	248	322	205	250	180	225	235	280	155	190	120
2	248	258	215	220	200	185	185	258	120	215	145	190	210	180	120
3	275	402	225	120	275	195	200	220	217	185	335	250	235	190	170
4	270	400	385	240	225	200	200	252	255	268	235	220	300	130	170
5	195	230	335	272	200	210	190	340	205	222	295	160	230	235	155
6	240	348	335	296	185	250	185	235	160	170	225	235	250	132	140
7	275	280	325	390	200	215	210	175	212	142	270	240	220	120	270
8	218	335	400	370	235	340	260	310	305	160	200	200	335	232	100
9	260	415	430	365	230	220	245	370	232	232	222	215	235	150	130
10	275	375	360	340	240	250	160	375	257	232	340	260	260	190	210
11	335	392	305	300	200	232	225	345	210	280	180	185	310	280	245
12	345	380	360	320	265	255	220	420	200	155	250	255	235	260	200
13	270	310	415	385	250	262	230	290	190	280	320	220	290	160	240
14	155	395	355	398	330	265	255	350	172	260	328	190	280	255	295
15	295	318	305	408	215	247	235	400	182	280	315	245	345	140	250
16	335	185	422	190	220	210	155	335	130	265	245	300	208	140	220
17	242	280	295	305	225	210	295	345	132	258	215	132	130	135	205
18	305	310	400	312	450	210	305	290	182	342	275	145	210	180	255
19	380	250	320	322	300	327	232	290	320	305	260	165	140	140	190
20	318	367	355	225	348	232	230	320	150	345	290	265	265	160	230
21	347	412	280	300	290	205	287	315	185	355	220	240	280	180	295
22	260	308	305	230	230	205	315	385	185	225	257	222	237	200	230
23	264	308	280	270	310	235	265	380	285	227	235	200	267	165	250
24	248	318	280	270	205	275	320	328	245	192	180	200	235	185	240
25	210	315	265	260	155	200	415	370	255	170	150	190	245	245	115
26	230	302	180	270	170	200	335	312	315	260	100	187	155	150	82

Total sum of squares of all individual yields

$$\Sigma p^2 = 26741531.$$

$$\text{Mean of ultimate plots or } \bar{p} = \frac{98217}{390} = 251.838 \text{ gms.}$$

TABLE LXV

*Yield of wheat in 1 × 5 combination plots*

Row No.	YIELD IN GMS. IN BLOCK No.		
	I	II	III
1 . . . . .	1033	1182	980
2 . . . . .	1141	963	845
3 . . . . .	1297	1017	1180
4 . . . . .	1520	1175	1055
5 . . . . .	1232	1167	1075
6 . . . . .	1404	1000	982
7 . . . . .	1470	954	1120
8 . . . . .	1558	1375	1067
9 . . . . .	1700	1299	952
10 . . . . .	1590	1274	1260
11 . . . . .	1532	1292	1200
12 . . . . .	1670	1250	1200
13 . . . . .	1630	1252	1230
14 . . . . .	1633	1302	1348
15 . . . . .	1541	1344	1295
16 . . . . .	1352	1095	1113
17 . . . . .	1347	1240	817
18 . . . . .	1777	1329	1065
19 . . . . .	1572	1474	895
20 . . . . .	1613	1277	1210
21 . . . . .	1629	1347	1215
22 . . . . .	1333	1315	1146
23 . . . . .	1432	1392	1117
24 . . . . .	1321	1360	1040
25 . . . . .	1205	1410	945
26 . . . . .	1152	1422	674
Total sum of squares	55569496	+41160675	+30845360

$$\text{i.e., } \Sigma Cp^2 = 127575531$$



TABLE LXVI

*Yield of wheat in 2 × 5 combination plots*

Row No.	YIELD IN GMS. IN BLOCK No.		
	I	II	III
1 and 2 . . . . .	2174	2145	1825
3 and 4 . . . . .	2817	2192	2235
5 and 6 . . . . .	2636	2167	2057
7 and 8 . . . . .	3028	2329	2187
9 and 10 . . . . .	3290	2573	2212
11 and 12 . . . . .	3202	2542	2400
13 and 14 . . . . .	3263	2554	2578
15 and 16 . . . . .	2893	2439	2408
17 and 18 . . . . .	3124	2569	1882
19 and 20 . . . . .	3185	2751	2105
21 and 22 . . . . .	2962	2662	2361
23 and 24 . . . . .	2753	2752	2157
25 and 26 . . . . .	2357	2832	1619
Total sum of squares . . . . .	110684070	+81927483	+61258640

*i.e.*,  $\Sigma Cp^2 = 253870193$ .

The coefficient of correlation between the fertility of contiguous plots is calculated by the formula—

$$r_{p,p_1} = \frac{[\sum (Cp^2) - \sum (p^2)\} / m \{n(n-1)\}] - (\bar{p})^2}{\sigma p^2} \quad . \quad . \quad . \quad (44)$$

where  $\bar{p}$  = average yield of all ultimate units ;

$n$  = number of units in each group ;

$m$  = number of groups ;

$\sum (p^2)$  = sum of squares of the yields assigned for ultimate units ;

$\sum (Cp^2)$  = sum of squares of the group yields ;

$\sigma p^2$  = square of the standard deviation of assigned yield for the ultimate units.

$$\sigma_p = \sqrt{\frac{\sum (p^2)}{n} - (\bar{p})^2} . . . . . (45)$$

*For 1 × 5 combination—*

$$r_{p_1 p_2} = \frac{[\{ 127575531 - 26741531 \} / 78 \{ 5 (5-1) \}] - (251.838)^2}{\left\{ \sqrt{\frac{26741531}{390}} - \left( \frac{98217}{390} \right)^2 \right\}^2} = 0.2361$$

$$S.E.r. = \frac{\pm (1 - r^2)}{\sqrt{n}} = \pm 0.0468$$

$$\therefore r_{p,p_2} = 0.2361 \pm 0.0468$$

For  $2 \times 5$  combination—

$$r_{p,p_2} = \frac{[\{253870193 - 26741531\} / 39 \{10(10 - 1)\}] - (251.838)^2}{\left\{ \sqrt{\frac{26741531}{390}} - \left( \frac{98217}{390} \right)^2 \right\}^2} = 0.2500$$

$$S.E.r = \frac{1-r^2}{\sqrt{n}} = 0.04747.$$

$$r_{p_1 p_2} = 0.2500 \pm 0.0475$$

## (2) FISHER'S ANALYSIS OF VARIANCE

For the sake of convenience, let us assume that we have 24 ultimate plots in each column instead of the actual 26 small plots. There are thus  $24 \times 15$  or 360 ultimate plots to consider in this field. The total sum of squares for calculating

the analysis of variance in the experiment is obtained by squaring the yield of each ultimate plot, summing and subtracting the product of the general total and the general mean. The sum of squares "between columns" is determined by squaring the total yield of each of the combination sub-plots or columns, summing, and dividing by the number of ultimate plots contributing to each of these combination sub-plots or columns, and subtracting the same product of the general total and the general mean as was used in obtaining the true total sum of squares. The sum of squares due to variations "within the columns" is, therefore, the difference between the total sum of squares and the sum of squares due to variation "between columns". As there are 360 ultimate plots in the whole experiment, the total degrees of freedom will be 359. The degrees of freedom "between columns" will be  $15 - 1$  or 14 and those "within columns" will be  $359 - 14$  or 345, in other words, the degrees of freedom "within columns" are  $23 \times 15$ . The mean squares or variance for each item can now be calculated by dividing the respective sum of squares by their appropriate degrees of freedom. The significance of the differences between and within columns can now be determined by Fisher's  $z$ -test or more easily by a modification of this test—Mahalanobis'  $x$ -test—which is nothing but a comparison of the ratio of variance<sub>1</sub> to variance<sub>2</sub>.

The following yields were obtained per column, each column consisting of 24 small plots, one square yard each, in area :—

Column No.	Yield in grms.
1	6425
2	7796
3	7952
4	7078
5	6076
6	5757
7	5609
8	7578
9	4911
10	5820
11	6072
12	5214
13	5862
14	4329
15	4930
<hr/>	
General total	. 91409
General mean	. $91409/360 = 253.914$

$$\text{Correction factor} = 91409 \times 253.914 = 23210024.826$$

$$\begin{aligned} \text{Total sum of squares} &= 25008515 - (\text{correction factor}) \\ &= 1798490.174 \end{aligned}$$

$$\begin{aligned} \text{Sum of squares between columns (squares of yields of each column summed} \\ \text{together)} \div 24 \text{ (the number of ultimate units in each column)} &= \frac{573791905}{24} \\ - (\text{correction factor}) &= 23907996.04 - (\text{correction factor}) = 697971.214. \end{aligned}$$

TABLE LXVII  
*Analysis of variance*

Variance	Degrees of freedom	Sum of squares	Mean squares	Mahalanobis' $x$ .
Between columns	14	697971.214	49855.0864	15.629
Within columns	345	1100518.960	3189.9100	
TOTAL	359	1798490.174	..	..

The expected ratio  $x$  [Mahalanobis, 1933] for  $n_1=12$  and  $n_2 = \infty$  is 2.182 for  $P = 0.01$ . Hence the observed ratio of 15.629 in this experiment is definitely significant.

From this it may be concluded that the variance between the 15 columns in this field is much greater than the variance within columns. In other words, there is a greater fertility difference between the columns from West to East than within them, *i.e.*, from North to South, a conclusion which is again in conformity with those obtained by Harris' method in the previous pages.

#### THE ANALYSIS OF COVARIANCE

If the results of uniformity trials such as described above are available for a particular field, or if the yielding capacities of sub-plots in a field in a preliminary trial be known it is possible sometimes to increase the precision of the experimental results obtained in any subsequent experiment, conducted in the same field, by the application of the analysis of covariance.

We have seen that Fisher's analysis of variance and its application to latin squares or randomized blocks, is one of the best methods of interpreting field results. The variance due to soil differences, from rows or from columns or that from blocks, being eliminated in this method, the residual variance, that is, that due to the chance errors of the experiment, furnishes a better criterion for estimating significance than the standard deviation calculated without any such elimination. In the words of Fisher [1932]—

“ the real and apparent precision of the comparison is the same as if the experiment had been performed on land in which the entire rows, and also the entire columns, were of equal fertility ”.

Another step forward was taken by Sanders [1930] in suggesting the application of the analysis of covariance to results of field trials. This method endeavours to increase the precision of experimental results on the basis of a knowledge of the yielding capacities of the same plots in a previous or preliminary trial. Sanders tried to determine whether soil variations were sufficiently constant, from year to year, to give useful corrections in the yields of experimental plots, from the yields of the same plots under previous uniformity trials. Considering the published results of uniformity trials with cereals, carried out on two fields at Aarslev (Denmark), during the years 1906 to 1911, he found that while in one field the precision of the experiment was increased by 150 per cent by utilising the previous

records, and correcting yields by the application of the method of covariance, yet in another field the plots showed no constancy in yield and he concluded that previous uniformity trials could not give any assistance in such a case. Eden [1931], however, obtained results of increased precision with a perennial crop, tea, by correcting experimental yields on the basis of previous croppings. In the case of perennial crops the same plants may be used in both the preliminary test and in the actual experiment and in such cases the analysis of covariance appears to possess advantages which are not present in its application to annual crops.

Fisher does not recommend, in general, the cropping of the experimental plots in the year previous to the experiment as it involves double the labour of the experiment and a year's delay before the result is made available. He states that "it seems, therefore, to be always more profitable to lay down an adequately replicated experiment on untried land than to expend time and labour in exploring irregularities of its fertility", and admits that "the chief advantage of the analysis of covariance lies not in its power of getting the most out of an existing body of data, but in the guidance it is capable of giving in the design of an observational programme, and in the choice of which many concomitant observational programmes shall in fact be recorded". In plant-breeding stations where the growing of bulk crops in fields which may be used for future yield trials is a necessary feature, it is however possible to secure, at little expense, an idea of soil-heterogeneity and to utilise the result for the correction of future yields.

Vaidyanathan [1933] has recently found that, by using preliminary yields in the case of a manurial experiment on tea, an improvement in the precision in the experiment is definitely brought out and that such preliminary yields can be utilized for designing an improved lay-out for future experiments. Similarly in the design of experiments on sugarcane in Padegaon Farm (Bombay Presidency) where yield figures of a previous crop of sunn-hemp, subjected to uniform treatment, were available, he found that these data might be utilised to the best advantage for designing subsequent experiments on sugarcane.

As an example of the analysis of covariance we may take the data of Pusa 52 wheat yields given in Table LXIV and data of the yields of type 21 barley in the preceding season in the same field.

*Example 24.*—The application of the analysis of covariance to the yields of annual crops, barley and wheat.

In this experiment there are 390 ultimate plots arranged in 26 rows and 15 columns. For the purpose of the analysis of covariance these ultimate units are combined to form a  $5 \times 5$  latin square, the 26th row being discarded from the experiment and the ultimate units combined in groups  $3 \times 5$ . Thus in Table LXIV the yields of 15 ultimate plots in rows 1 to 5 and columns 1 to 3 form the first sub-plot of the latin square. The yields of the sub-plots forming the latin square with barley are given in Table LXVIII and the yields of ultimate units of Pusa 52 wheat are given in Table LXIV and those of sub-plots forming the latin square in Table LXXIII. The various steps in the calculation of the analysis of variance for the preliminary series with barley are shown in Tables LXIX to LXXII.

TABLE LXVIII

*Preliminary yields (barley type 21)**(x-table)**Plot yields in grms.*

Rows	COLUMNS					Total
	A	B	C	D	E	
I . . .	1081.5	888.5	813.0	840.5	725.0	4348.5
II . . .	1218.5	1035.0	973.5	817.0	731.0	4775.0
III . . .	1216.0	1076.5	1055.5	825.0	693.5	4866.5
IV . . .	1213.5	1091.5	1026.0	921.5	736.5	4989.0
V . . .	1147.0	1068.0	1067.5	764.5	606.5	4653.5
TOTAL .	5876.5	5159.5	4935.5	4168.5	3492.5	23632.5

$$\text{Mean yield} = \frac{23632.5}{25} = 945.3 \text{ grms.}$$

TABLE LXIX

*Deviations from the mean, 945.3 grms.*

Rows	COLUMNS					Total
	A	B	C	D	E	
I . . .	+136.2	—56.8	—132.3	—104.8	—220.3	—378.0
II . . .	+273.2	+89.7	+28.2	—128.3	—214.3	+48.5
III . . .	+270.7	+131.2	+110.2	—120.3	—251.8	+140.0
IV . . .	+268.2	+146.2	+80.7	—23.8	—208.8	+262.5
V . . .	+201.7	+122.7	+122.2	—180.8	—338.8	—73.0
TOTAL .	+1150.0	+433.0	+209.0	—558.0	—1234.0	0

TABLE LXX

*Squares of deviations**( $x^2$ -table)*

Rows	COLUMNS					Total
	<i>A</i>	<i>B</i>	<i>C</i>	<i>D</i>	<i>E</i>	
I . .	18550.44	3226.24	17503.29	10983.04	48532.09	98795.10
II . .	74638.24	8046.09	795.24	16460.89	45924.49	145864.95
III . .	73278.49	17213.44	12144.04	14472.09	63403.24	180511.30
IV . .	71931.24	21374.44	6512.49	566.44	43597.44	143982.05
V . .	40682.89	15055.29	14932.84	32688.64	114785.44	218145.10
TOTAL .	279081.30	64915.50	51887.90	75171.10	316242.70	787298.50

TABLE LXXI

*Sum of squares of preliminary yields*

Rows		Columns		
<i>d</i>	<i>d</i> <sup>2</sup>	<i>d</i>	<i>d</i> <sup>2</sup>	
—378.0	142884.00	+1150.0	1322500.00	Total sum of squares 787298.50.
+48.5	2532.25	+433.0	187489.00	
+140.0	19600.00	+209.0	43681.00	
+262.5	68906.25	—558.0	311364.00	
—73.0	5329.00	—1234.0	1522756.00	
TOTAL .	239071.50	..	3387790.00	
Divided by 5 .	47814.30	..	677558.00	

TABLE LXXII.

*Analysis of variance of preliminary yields*

Due to	Degrees of freedom	Sums of squares	Mean squares
Rows . . . . .	4	47814.30	11953.575
Columns . . . . .	4	677558.00	169389.500
Error . . . . .	16	61926.20	3870.3875
TOTAL .	24	787298.50	32804.104

Similarly, the plot yields in the experimental series with Pusa 52 wheat are shown in Table LXXIII and the subsequent tables, *viz.*, Tables LXXIV to LXXVII show the various stages in the calculation of the analysis of variance for this series.

TABLE LXXIII

*Experimental yields in Pusa 52 wheat**(y-table)**Plot yields in grms.*

Rows	COLUMNS					Total
	A	B	C	D	E	
I . . .	309.3	334.2	327.7	346.0	279.0	1686.2
II . . .	487.1	412.6	369.1	334.3	297.4	1900.5
III . . .	493.5	433.2	392.4	374.3	378.5	2071.9
IV . . .	476.4	408.6	371.1	380.7	280.8	1917.6
V . . .	440.0	364.0	453.5	326.3	336.9	1920.7
TOTAL .	2296.3	1952.6	1913.8	1761.6	1572.6	9496.9

$$\text{Mean yield} = \frac{9496.9}{25} = 379.876 \text{ grms.}$$



TABLE LXXIV

*Deviations from arbitrary mean of 380 grms.*

Rows	COLUMNS					TOTAL
	<i>A</i>	<i>B</i>	<i>C</i>	<i>D</i>	<i>E</i>	
I . . .	+19.3	—45.8	—52.3	—34.0	—101.0	—213.8
II . . .	+107.1	+32.6	—10.9	—45.7	—82.6	+0.5
III . . .	+113.5	+53.2	+12.4	—5.7	—1.5	+171.9
IV . . .	+96.4	+28.6	—8.9	+0.7	—99.2	+17.6
V . . .	+60.0	—16.0	+73.5	—53.7	—43.1	+20.7
TOTAL .	+396.3	+52.6	+13.8	—138.4	—327.4	—3.1

TABLE LXXV

*Squares of deviations from arbitrary mean ( $y^2$ -table)*

Rows	COLUMNS					TOTAL
	<i>A</i>	<i>B</i>	<i>C</i>	<i>D</i>	<i>E</i>	
I . . .	372.49	2097.64	2735.29	1156.00	10201.00	16562.42
II . . .	11470.41	1062.76	118.81	2088.49	6822.76	21563.23
III . . .	12882.25	2830.24	153.76	32.49	2.25	15900.99
IV . . .	9292.96	817.96	79.21	0.49	9840.64	20031.26
V . . .	3600.00	256.00	5402.25	2883.69	1857.61	13999.55
TOTAL .	37618.11	7064.60	8489.32	6161.16	28724.26	88057.45

TABLE LXXVI

*Sum of squares of experimental yields*

Rows		Columns		
<i>d</i>	<i>d</i> <sup>2</sup>	<i>d</i>	<i>d</i> <sup>2</sup>	
—213.8	45710.44	+396.3	157053.69	Crude sum of squares = 88057.45
+0.5	0.25	+52.6	2766.76	
+171.9	29549.61	+13.8	190.44	Correction for average
				$= \frac{(3.1)^2}{25} = 0.384$
+17.6	309.76	—138.4	19154.56	
+20.7	428.49	—327.4	107190.76	True total sum of squares = 88057.0656
TOTAL .	75998.55	..	286356.21	
Divided by 5 .	15199.71	..	57271.242	
Subtract correction	0.3844	..	0.3844	
Sum of squares .	15199.3256	..	57270.8576	

TABLE LXXVII

*Analysis of variance of experimental yields*

Due to								Degrees of freedom	Sum of squares	Mean squares
Rows	.	.	.	.	.	.	.	4	15199.3256	3799.8314
Columns	.	.	.	.	.	.	.	4	57270.8576	14317.7144
Error	.	.	.	.	.	.	.	16	15586.8824	974.18015
TOTAL .								24	88057.0656	3669.0444

We note that the residual error in the case of the preliminary series (Pusa barley type 21) is 3870.3875 and that in the experimental series (Pusa 52 wheat) the residual error is 974.1804 only.

To obtain an estimate for correcting the residual error of the experimental series on the basis of the error of the preliminary series we use the method of covariance. The principle involved is the use of the regression of experimental yields

on previous yields for all the plots concerned. Using the regression equation of the form

$$y = b.x$$

where  $y$  = the yield in the experimental series and  $x$  = the previous yield; a new variance of  $y$  corrected for  $x$  is obtained and this new variance satisfies the equation:—

$$V_{y.x} = V_y (1 - r^2_{xy}) = V_y - \frac{(\text{Cov. } xy)^2}{V_x} \dots \dots \dots (46)$$

where  $\text{Cov. } xy$  is the covariance between  $x$  and  $y$ . In other words COVARIANCE may be defined as the mean product of deviations of the two variates just as VARIANCE is the mean product of the deviations of a single variate. If the produce of an individual plot in one year is any guide to its performance in another, the variance  $V_{y.x}$  will naturally give the variance of  $y$  corrected by the regression equation

$$y = b.x$$

$$\text{where } b = \frac{\text{Cov. } xy}{V_x}$$

corrected mean plot yields given by any two treatments will be the difference between two expressions of the form  $\frac{\sum (y - bx)}{n}$

If  $n$  be the number of replicates the difference between the

Table LXXVIII shows the calculation of the term  $\sum xy$ . This is done by obtaining the products of deviations in Tables LXIX and LXXIV and summing them.

TABLE LXXVIII

*Products of deviations taken from tables LXIX and LXXIV (XY-table)*

Rows	COLUMNS					TOTAL XY
	A	B	C	D	E	
I . .	+2628.66	+2601.44	+6919.29	+3563.20	+22250.30	+37962.89
II . .	+29259.72	+2924.22	—307.38	+5863.31	+17701.18	+55441.05
III . .	+30724.45	+6979.84	+1366.48	+685.71	+377.70	+40134.18
IV . .	+25854.48	+4181.32	—718.23	—16.66	+20712.96	+50013.87
V . .	+12102.00	—1963.20	+8981.70	+9708.96	+14602.28	+43431.74
TOTAL .	+100569.31	+14723.62	+16241.86	+19804.52	+75644.42	+226983.73

The XY products for rows and columns respectively are determined by multiplying the totals for rows or for columns in the preliminary (X) series by the corresponding totals in the experimental (Y) series and summing them.

The sums of squares for rows and columns of the X and Y series have already been determined in Tables LXXI and LXXVI but for convenience may again be included in Table LXXIX in conjunction with the XY values.

TABLE LXXIX

*Analysis of covariance*

	ROWS					COLUMNS				
	X	X <sup>2</sup>	XY	Y	Y <sup>2</sup>	X	X <sup>2</sup>	XY	Y	Y <sup>2</sup>
I . . . . .	-378.0	..	+80816.40	-213.8	..	+1150.0	..	+455745.00	+396.3	..
II . . . . .	+48.5	..	+24.25	+0.5	..	+433.0	..	+22775.80	+52.6	..
III . . . . .	+140.0	..	+24066.00	+171.9	..	+209.0	..	+2884.20	+13.8	..
IV . . . . .	+262.5	..	+4620.00	+17.6	..	-558.0	..	+77227.20	-138.4	..
V . . . . .	-73.0	..	-1511.10	+20.7	..	-1234.0	..	+404011.60	-327.4	..
Total . . . . .	..	239071.50	+168015.55	..	75998.55	..	3387790.00	962643.80	..	286356.21
Mean . . . . .	..	47814.30	+21603.11	..	15199.71	..	677558.00	192528.76	..	57271.242
Subtract correction factor	..	..	..	..	0.3844	..	..	..	..	0.3844
True sum of squares .	..	47814.30	21603.11	..	15199.3256	..	677558.00	192528.76	..	57270.8576

Table LXXX summarises these results and in addition has the  $X^2$ ,  $XY$  and  $Y^2$  values for residual error, these values being obtained simply by deducting the sums of squares for  $X$  and  $Y$  and the sums of  $XY$  products for rows and columns from the totals.

TABLE LXXX

*Sums of squares and products*

Due to	Degrees of freedom	$X^2$	$XY$	$Y^2$
Rows . . .	4	47814.30	21603.11	15199.3256
Columns . . .	4	677558.00	192528.76	57270.8576
Error . . .	16	61926.20	12851.86	15586.8868
TOTAL .	24	787298.50	226983.73	88057.0700

We have now to calculate the coefficient of regression by dividing the sum of products for error of the  $xy$  value by the sum of squares for error of the preliminary yields. Thus

$$b = \frac{\text{Cov. } xy}{V_x} = \frac{12851.86}{61926.20} = 0.2075.$$

This correction may be applied either to individual plots or to the composite totals represented by rows, columns, treatments or errors.

To obtain sums of squares for adjusted yields in any line (*i.e.*, either in rows, columns or in errors) we multiply the entries under  $x^2$ ,  $xy$  and  $y^2$  in that particular line in Table LXXX respectively by the values of  $b^2$ ,  $-2b$  and 1 as shown below and add the products and tabulate them as shown in Table LXXXI.

We see that the coefficient of regression,  $b = 0.2075$ , therefore  $b^2 = 0.0431$  and  $2b = 0.4150$ . The adjusted sums of squares, therefore, are:—

For rows  $(47814.30 \times 0.0431) + 21603.11 \times (-0.4150) + 15199.3256 = 8294.83128$ .

For columns  $(677558.00 \times 0.0431) + 192528.76 \times (-0.4150) + 57270.8576 = 6574.1720$ .

For error  $(61926.20 \times 0.0431) + 12851.86 \times (-0.4150) + 15586.8868 = 12922.3841$ .

The following table shows the analysis of adjusted yields.

TABLE LXXXI

*Analysis of adjusted yields*

Due to	Degrees of freedom	Sum of squares	Mean squares
Rows . . . .	4	8294.8313	2073.7078
Columns . . . .	4	6574.1720	1643.5430
Error . . . .	15	12922.3841	861.4923
Regression . . . .	1	2664.5027	2664.5027

Comparing this analysis of adjusted yields with the analysis of variance for experimental yields shown in Table LXXVII, the most striking change observed is the reduction of the mean square for error from 974.18015 to 861.4923, in spite of the reduction in the degrees of freedom appropriate to it, showing thereby that the precision of the comparison has been increased by taking the preliminary yields into consideration.

Thus—

$$\frac{\text{Mean square for error in Table LXXVII}}{\text{Mean square for error in Table LXXXI}} = \frac{974.180}{861.492} = 1.1308.$$

In this case it may be pointed out that the ratio between the two mean squares considered is only 1.13.

In perennial crops such as tea, Eden [1931] has secured an increased precision of 6.81 times in comparison with the experimental error existing without the use of the analysis of covariance. Recently, Vaidyanathan [1934] has similarly obtained an improvement of about 16 times in the precision of tea results of the Tocklai Tea Experimental Station by using preliminary data.

It may be pointed out that the application of the method of covariance for correcting experimental yields on the basis of the previous cropping gives more satisfactory results in the case of perennial crops, *e.g.*, tea, than in the case of annuals, *e.g.*, wheat, barley, etc. Vaidyanathan's results with the tea figures obtained from the Tocklai Station may be taken as an example of the application of the method of covariance in improving the precision of results in perennial crops.

*Example 25.* The application of analysis of covariance to tea results. This experiment was carried out in 6 blocks with 4 plots in each block.

Table LXXXII shows the preliminary yields of tea obtained at Tocklai, and during this preliminary period hypothetical treatments (*A*, *B*, *C*, *D*) have been

assumed to exist and to be distributed in the field on the same lay-out as during the final experiment.

TABLE LXXXII

*Preliminary yields of tea (non-manurial treatment) (X-table)*

Plot yields

Blocks	A	B	C	D	TOTAL
I . . . .	134	118	99	104	455
II . . . .	133	105	138	142	518
III . . . .	103	129	129	143	504
IV . . . .	104	126	104	79	413
V . . . .	143	127	142	127	539
VI . . . .	109	113	111	127	460
TOTAL .	716	718	723	722	2889

The analysis of variance of the preliminary yields is shown in Table LXXXIII

TABLE LXXXIII

*Analysis of variance of preliminary yields*

Due to	Degrees of freedom	Sum of squares	Mean square
Blocks . . . .	5	2750.375	550.75
Hypothetical treatments . .	3	5.458	1.82
Error . . . .	15	3959.792	263.99
TOTAL .	23	6715.625	291.98

The experimental yields are shown in Table LXXXIV shown under :—

TABLE LXXXIV

*Experimental yields of tea (with 4 manurial treatments in the experiment) (Y-table)*

## Plot yields

Blocks	TREATMENTS				TOTAL
	A	B	C	D	
I . . . .	135	110	98	117	460
II . . . .	136	107	145	175	563
III . . . .	102	125	138	181	546
IV . . . .	108	136	114	96	454
V . . . .	149	116	164	144	573
VI . . . .	110	110	120	152	492
TOTAL .	740	704	779	865	3088

The analysis of variance of this series is shown in Table LXXXV given below :—

TABLE LXXXV

*Analysis of variance of experimental yields*

Due to	Degrees of freedom	Sum of squares	Mean square
Blocks . . . .	5	3475.83	695.17
Treatments . . . .	3	2391.00	797.00
Error . . . .	15	7222.50	481.50
TOTAL .	23	13089.33	569.10

The various steps of calculation have been shown in great detail in the previous example and have, therefore, been omitted in the present case. The analysis of adjusted yields is shown in Table LXXXVI.



TABLE LXXXVI

*Analysis of variance of adjusted yield ( $y-bx$ ):*

Due to	Degrees of freedom	$y^2$	$b^2x^2$	$-2bxy$	$y^2+b^2x^2-2bxy$	Mean square
Block . .	5	3475.83	2750.37 $\times 1.714$	-2978.75 $\times 2.6184$	390.414	78.08
Treatments .	3	2391.00	5.46 $\times 1.714$	-25.17 $\times 2.6184$	2334.450	778.15
Error. . .	14 (after allowing 1 degree for linear regression).	7222.50	3959.79 $\times 1.714$	-5184.18 $\times 2.6184$	435.588	31.11
TOTAL .	22	..	..	..	3160.452	

Improvement in precision by using the preliminary data

$$= \frac{\text{Mean square for 'error' in Table LXXXV}}{\text{Mean square for 'error' in Table LXXXVI}} = \frac{481.5}{31.11} = \text{about 15.5 times.}$$

The above table of adjusted yield obtained after allowing for linear regression shows that by combining the 'preliminary' and 'experimental' analysis the standard error of the experiment is considerably reduced, and that the improvement in precision is nearly 16 times what it would otherwise be by analysing the experimental data alone. Where preliminary yields of experimental plots can be secured, it is possible, therefore, to obtain greater precision of results by applying the method of covariance.

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## APPENDIX I

### LOGARITHMS

The ordinary processes of arithmetical computations are greatly simplified by the use of logarithms. Logarithms are a practical application of the well-known algebraic rule

$$x^2 \times x^3 = x^{2+3} = x^5$$

$$\text{and } x^5 \div x^3 = x^{5-3} = x^2.$$

It is possible to express any number as a power of another number, *e.g.*,

$$64 = 8^2 = 4^3.$$

In this case, 64 is the square or second power of 8 and the cube or third power of 4. If we desired to express 64 as a power of 10 we should get an index (power) of less than 2, since 10 is larger than 8. Actually it is found by calculation that  $64 = 10^{1.8062}$  and 1.8062 is called the logarithm of 64 to the base 10.

Logarithms of numbers are powers to the base 10, thus—

$$100 = 10^2, \text{ therefore log. } 100 = 2.0.$$

$$1,000 = 10^3, \text{ therefore log. } 1000 = 3.0.$$

$$10,000 = 10^4, \text{ therefore log. } 10000 = 4.0.$$

Tables of logarithms have been constructed by mathematicians for all numbers up to 10,000 and are of enormous use in shortening the processes of calculation. The logarithm of a number consists of an integral part called the *characteristic* and a decimal part, the *mantissa*.

For example, from a table of logarithms we find that the logarithm of 5184.0 is 3.7147. In this case 3 is the *characteristic* and 0.7147 is the *mantissa*. In the case of numbers greater than unity the characteristic is always one less than the number of figures to the left of the decimal point. Thus—

The logarithm of a number less than unity is negative but the mantissa is kept positive by making the characteristic negative and 1 more than the number of zeros which follow the decimal point.

$$\begin{aligned} \log. 5184.0 &= 3.7147. \\ \log. 518.4 &= 2.7147. \\ \log. 51.84 &= 1.7147. \\ \log. 5.184 &= 0.7147. \end{aligned}$$

Thus—

$$\begin{aligned} \log. 0.5184 &= -1 + 0.7147 \text{ or } \bar{1}.7147 \\ \log. 0.05184 &= -2 + 0.7147 \text{ or } \bar{2}.7147 \end{aligned}$$

If we have to multiply  $64 \times 81$  by the use of logarithms in practice we proceed as follows:—

$$\begin{aligned} \log. 64 &= 1.8062. \\ \log. 81 &= 1.9085 \end{aligned}$$

$$\therefore \log. (64 \times 81) = 3.7147$$

From a table of antilogarithms we can find the number corresponding to a given logarithm. In this way we identify 7147 as the logarithm of 5184, and since the characteristic in this case is 3 the number required must be between  $10^3$  and  $10^4$ , *i.e.*, between 1,000 and 10,000. Therefore  $64 \times 81 = 5184.0$ .

In the case of division

$$\begin{aligned} \frac{81}{64} \text{ we get } \log. \frac{81}{64} &= \log 81 - \log 64 \\ \text{or } (1.9085 - 1.8062) &= 0.1023 \\ \text{but } 0.1023 &= \log. 1.266 \\ \text{i.e. } \frac{81}{64} &= 1.266. \end{aligned}$$

*Example 1.*

$$\begin{aligned} &\text{Multiply } 47.5620 \text{ by } 0.003710 \\ &\log. \quad 47.5620 = 1.67726 \\ &\text{and } \log. \quad 0.003710 = \bar{3}.56937 \end{aligned}$$

$$\begin{aligned} \therefore \log. (47.5620 \times 0.003710) &= \bar{1}.24663 \\ &= \log. 0.17645 \\ \text{i.e., } 47.5620 \times 0.003710 &= 0.17645. \end{aligned}$$

*Example 2.*

$$\begin{aligned} &\text{Divide } 0.0516 \text{ by } 33.6210 \\ &\log. \quad 0.0516 = \bar{2}.71265 \\ &\log. \quad 33.6210 = 1.52661 \end{aligned}$$

$$\begin{aligned} \log. \frac{0.0516}{33.6210} &= \bar{3}.18604 \\ &= \log. 0.0015347 \end{aligned}$$

$$\text{i.e., } \frac{0.0516}{33.6210} = 0.0015347$$

*Example 3.*

$$\begin{aligned} &\text{Divide } 0.0176 \text{ by } 0.008437 \\ &\log. \quad 0.0176 = \bar{2}.24550 \\ &\log. \quad 0.008437 = \bar{3}.92619 \end{aligned}$$

$$\begin{aligned} \log. \frac{0.0176}{0.008437} &= 0.31931 \\ &= \log. 2.08595 \end{aligned}$$

$$\text{i.e., } \frac{0.0176}{0.008437} = 2.08595$$

*Example 4.*

$$\begin{aligned} &\text{Divide } 0.008437 \text{ by } 0.0176 \\ &\log. \quad 0.008437 = \bar{3}.92619 \\ &\log. \quad 0.0176 = \bar{2}.24550 \end{aligned}$$

$$\begin{aligned} \log. \frac{0.008437}{0.0176} &= \bar{1}.68069 \\ &= \log. 0.4794 \end{aligned}$$

$$\text{i.e., } \frac{0.008437}{0.0176} = 0.4794$$

*Example 5.*

$$\begin{aligned} &\text{Divide } 0.0176 \text{ by } 0.0516 \\ &\log. \quad 0.0176 = \bar{2}.24550 \\ &\log. \quad 0.0516 = \bar{2}.71265 \end{aligned}$$

$$\begin{aligned} \log. \frac{0.0176}{0.0516} &= \bar{1}.53285 \\ &= \log. 0.34108 \end{aligned}$$

$$\text{i.e., } \frac{0.0176}{0.0516} = 0.34108$$

The calculations of square roots and the powers of numbers are greatly simplified by the use of logarithms. Students must remember that

$$\begin{aligned}\log. a^n &= n \log. a \\ \text{i.e., } \log. (64)^2 &= 2. \log. 64 \\ &= 2 \times 1.8062 \\ &= 3.6124 \\ &= \log. 4096;\end{aligned}$$

and again that

$$\begin{aligned}a^{\frac{1}{2}} \times a^{\frac{1}{2}} &= a \\ \therefore \sqrt{a} &= a^{\frac{1}{2}} \\ \log. \sqrt{a} &= \frac{1}{2} \log. a \\ \text{or } \log. \sqrt{64} &= \frac{1}{2} \times 1.8062 \\ &= 0.9031 \\ &= \log. 8.\end{aligned}$$

Similarly

$$\begin{aligned}a^{\frac{1}{3}} \times a^{\frac{1}{3}} \times a^{\frac{1}{3}} &= a \\ \therefore \sqrt[3]{a} &= a^{\frac{1}{3}} \\ \log. \sqrt[3]{a} &= \frac{1}{3} \log. a \\ \text{and } (a^{\frac{1}{3}})^3 &= a^{\frac{1}{3}} = (\sqrt[3]{a})^3 \\ \therefore \log. (a^{\frac{1}{3}})^3 &= 3 \times \frac{1}{3} \log. a.\end{aligned}$$

The SLIDE RULE is a mechanical device of which the two principal components slide over one another and are each graduated in a logarithmic scale. This allows of the determination of simple products, quotients, and roots even more rapidly than with a table of logarithms. Students should familiarise themselves with the use of the slide rule. A 20-inch rule is accurate to four figures and is sufficient for most of the statistical computations met with in biological problems.

NAPERIAN LOGARITHMS.—The logarithms described above are calculated to the base 10 and are those which are universally employed for ordinary computations. In higher mathematics and in certain cases in statistics Napierian logarithms which are calculated to the base 2.71828 are used. It is not necessary here to give details of the theory of Napierian logarithms, it is sufficient to state that the number, 2.71828..... is an incommensurable number generally indicated by the symbol  $e$ , and is the summation of the series

$$1 + \frac{1}{1} + \frac{1}{1.2} + \frac{1}{1.2.3} + \frac{1}{1.2.3.4} + \dots \dots \dots \infty$$

which, when worked out to the first six decimal places, gives the result 2.718282...

Tables of logarithms to the base  $e$  are not always available but common logarithms to the base 10 can be converted into Napierian logarithms by a simple equation:—

$$\begin{aligned}\log._{10} a &= \log. e a \times 0.4343 \\ \log. e a &= \frac{\log._{10} a}{0.4343} = \log._{10} a \times 2.3025\end{aligned}$$

When  $a$  = any number.

The conversion figure 0.4343 is called the modulus of the common system of logarithms.

Example 6 shows the method of obtaining the logarithms to the base  $e$ , by the use of a table of Napierian logarithms, of a series of numbers in which the same digits occur with different decimal values.

*Example 6.*—

(a) Find  $\log_e 5476.12$ .

$$\log_e (5.47612 \times 1000) = \log_e 5.47612 + \log_e 10^3$$

$\log_e$  for the number 5.47612 is given by the summation below :—

$$\begin{array}{r} 1.69928 \\ 110 \\ 18 \\ 037 \\ \hline 1.7004017 \end{array}$$

From the table,  $\log_e 10^3 = 6.907760$

$$\text{Therefore } \log_e 5476.12 = 1.70040 + 6.90776 = 8.60816$$

(b) Find  $\log_e 547.612$

$\log_e$  for the number 5.47612 = 1.70040 as shown above.

$$\log_e 10^2 = 4.60517$$

$$\text{Therefore } \log_e 547.612 = 1.70040 + 4.60517 = 6.30557$$

(c) Find  $\log_e 54.7612$

$$\log_e 10^1 = 2.30258$$

$$\text{Therefore } \log_e 54.7612 = 4.00298$$

(d) Find  $\log_e 5.47612$

The required logarithm is ~~the logarithm of the number~~ 1.70040.

(e) Find  $\log_e 0.547612$

$$\log_e 10^{-1} = \bar{3}.697410$$

$$\log_e 5.47612 = 1.70040$$

$$\log_e 0.547612 = \bar{1}.39781$$

(f) Find  $\log_e 0.0547612$

$$\log_e 10^{-2} = \bar{5}.394830$$

$$\log_e \text{ of number} = 1.70040$$

$$\log_e 0.0547612 = \bar{3}.09523.$$

## INTERPOLATION

A function  $y = f(x)$  can be evaluated accurately, provided  $f(x)$  is an algebraic expression involving squaring, addition, subtraction, multiplication and division. But if  $y = \log x$ , the value of  $y$  cannot be so easily calculated. In such cases where the value of  $y$  cannot be got by the performance of a definite number of finite simple arithmetical operations, we are forced to have recourse to a table which gives the values of  $y$  corresponding to certain convenient values of  $x$ . Here we select a number of values  $x_1, x_2, \dots, x_n$  etc. for  $x$  and tabulate the corresponding values of  $y$ . Let us assume that the logarithms of numbers 1, 2, ..., 100 have been tabulated. The question arises as to how we can find the values of  $\log x$  intermediate between any two of the numbers of the table. The answer to this question is given by the theory of interpolation which in its elementary aspect is the science of "reading between the lines of a mathematical table".

Again it sometimes happens in statistical records that gaps occur which it is desirable to fill in. This may be due to lack of sufficient observations or to the destruction of old records. If we have a frequency distribution in which a few data are missing, we can by the method of interpolation supply the missing data to a fair degree of approximation.

A short example will suffice to illustrate the principle involved. Suppose we want to find the logarithm of 255.475. From logarithmic tables, we get that  $\log_{10} 255 = 2.40654$  and  $\log_{10} 256 = 2.40824$ . If the logarithms and the numbers are taken as the two variables and if we plot these on a graph paper, we will get a logarithmic curve. But for all practical purposes the portion of the curve between 255 and 256 can be considered to be a straight line and the value of the logarithm with respect to the base 10 for 255.475 can be written down by applying the simple method of proportions.

$$\log. 255.475 = 2.40654 + .475 \times \frac{(2.40824 - 2.40654)}{256 - 255} = 2.40654 + .0008075 = 2.4073475.$$

Now if we have got a table which gives the logarithms of 255.4 and 255.5 we can, by using the same method, get the value of  $\log. 255.475$ .

$$\log_{10} 255.4 = 2.40722$$

$$\log_{10} 255.5 = 2.40739$$

As before

$$\log. 255.475 = 2.40722 + .275 \times \frac{(2.40739 - 2.40722)}{255.5 - 255.4} = 2.40722 + .0001275 = 2.4073475.$$

It may be noted that the values of the  $\log. 255.475$  got by the two methods are identical in this case. But in some cases there may be slight differences, because the portion of the curve between the two values of  $x$  need not be a straight line. It may be a curve in which case some corrections will have to be applied. But for all practical purposes for using the logarithmic and other tables, we can assume that the corrections are so small that they can be neglected safely and the method indicated above, on the assumption that a linear relation exists between two variables for a particular small range, can be adopted for interpolation.

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 Feldman, W. M. (1923) Biomathematics. Charles Griffin and Co., London.  
 Rasor, S. E. (1923) Mathematics for Students of Agriculture. Macmillan, New York.  
 Thompson, J. E. (1931) A Manual of the Slide Rule: its History, Principle and Operation. Chapman and Hall, London.

# APPENDIX II

## IMPORTANT FORMULÆ

	Formula number	Page
. . . . .	(1)	9
. . . . .	(2)	14
samples		
$= \sqrt{\frac{\sum w_1 + \sum w_2}{(N_1-1) + (N_2-1)}}$	(24)	54
Aver. dev. = $\frac{\sum f d }{n}$	(3)	15
$C. V. = \frac{\sigma \times 100}{M}$	(4)	15
$P. E. \text{ Mean} = \frac{0.6745 \sigma}{\sqrt{n}}$	(7)	27
$P. E. \sigma = \frac{0.6745 \sigma}{\sqrt{2n}}$	(8)	27
$P. E. \text{ c. v.} = 0.6745 V \times \left\{ 1 + 2 \left( \frac{V}{100} \right)^2 \right\}^{\frac{1}{2}} / \sqrt{2n}$	(9)	28
$P. E. r = \frac{\pm 0.6745 (1-r^2)}{\sqrt{n}}$	(33)	67
$P. E. \text{ diff.} = \sqrt{E_1^2 + E_2^2}$	(10)	29
$S. E. d(1-2) = \sqrt{(S. E._1)^2 + (S. E._2)^2}$	(19)	48
$P. E. \text{ average} = \frac{1}{N} \sqrt{a^2 + b^2 + c^2 + \dots + n^2}$	(11)	31
$P. E. \text{ of any probability} = 0.6745 \sqrt{\frac{p \times q}{n}}$	(13)	32
$P. E. \text{ of a class frequency} = 0.6745 \sqrt{p \cdot q \cdot n.}$	(14)	32
$r_{xy} = \frac{\sum (f. \delta x. \delta y)}{N. \sigma x. \sigma y.}$	(32)	65
$r_{xy} = \frac{\frac{\sum (XY)}{n} - (\bar{X} \cdot \bar{Y})}{\sqrt{\frac{\sum (X^2)}{n} - (\bar{X})^2} \cdot \sqrt{\frac{\sum (Y^2)}{n} - (\bar{Y})^2}}$	(36)	38

	Formula number	Page
$\chi^2 = \frac{\Sigma (o - c)^2}{c}$ . . . . .	(15)	33
Student's $Z = \frac{\text{Md.}}{\sqrt{\frac{\Sigma d^2}{n}}}$ . . . . .	(21)	51
Fisher's $z = 1/2 \log_e \frac{v_1}{v_2}$ . . . . .	(One sample). ..	103
Fisher's $t = \frac{m_1 - m_2}{s \sqrt{\frac{1}{N_1} + \frac{1}{N_2}}}$ . . . . .	(23)	53
Mahalanobis' $x = \frac{\text{Variance}_1}{\text{Variance}_2}$ . . . . .	(two samples). ..	104
	$S$ being given by formula	
Mahalanobis' $f = \frac{m_1 - m_2}{\sqrt{\frac{(s_1)^2 + (s_2)^2}{2}}}$ . . . . .	(24) (30)	62
$f = t \sqrt{\frac{2}{n}}$ . . . . .	(31)	63
Critical difference $d = t \times S. E.$ . . . . .	(27)	58

Insert Formula (25)  $t =$

$$s \sqrt{\frac{1}{N_1} + \frac{1}{N_2}}$$



## APPENDIX III

SNEDCOR'S TABLES FOR THE VALUES OF  $F$  (RATIO OF VARIANCE<sub>1</sub> TO VARIANCE<sub>2</sub>)  
AND FISHER'S  $t$  FOR DIFFERENT DEGREES OF FREEDOM

	Degrees of freedom for greater mean square										Values of $t$
	1	2	3	4	5	6	8	12	24	$\infty$	
1	161.45 4052.10	199.50 4999.03	215.72 5403.49	224.57 5625.14	230.17 5764.08	233.97 5859.39	238.89 5981.34	243.91 6105.83	249.04 6234.16	254.32 6366.48	12.706 63.657
2	18.51 98.49	19.00 99.01	19.16 99.17	19.25 99.25	19.30 99.30	19.33 99.33	19.37 99.36	19.41 99.42	19.45 99.46	19.50 99.50	4.303 9.925
3	10.13 34.12	9.55 30.81	9.28 29.46	9.12 28.71	9.01 28.24	8.94 27.91	8.84 27.49	8.74 27.05	8.64 26.60	8.53 26.12	3.182 5.841
4	7.71 21.20	6.94 18.00	6.59 16.69	6.39 15.98	6.26 15.52	6.16 15.21	6.04 14.80	5.91 14.37	5.77 13.93	5.63 13.46	2.776 4.604
5	6.61 16.26	5.79 13.27	5.41 12.06	5.19 11.39	5.05 10.97	4.95 10.67	4.82 10.27	4.68 9.89	4.52 9.47	4.36 9.02	2.571 4.032
6	5.99 13.74	5.14 10.92	4.76 9.78	4.53 9.15	4.39 8.75	4.28 8.47	4.15 8.10	4.00 7.72	3.84 7.31	3.67 6.88	2.447 3.707
7	5.59 12.25	4.74 9.55	4.35 8.45	4.12 7.85	3.97 7.46	3.87 7.19	3.73 6.84	3.57 6.47	3.41 6.07	3.23 5.65	2.365 3.499
8	5.32 11.26	4.46 8.65	4.07 7.59	3.84 7.01	3.69 6.63	3.58 6.37	3.44 6.03	3.28 5.67	3.12 5.28	2.93 4.86	2.306 3.355
9	5.12 10.56	4.26 8.02	3.86 6.99	3.63 6.42	3.48 6.06	3.37 5.80	3.23 5.47	3.07 5.11	2.90 4.73	2.71 4.31	2.262 3.250
10	4.96 10.04	4.10 7.56	3.71 6.55	3.48 5.99	3.33 5.64	3.22 5.39	3.07 5.06	2.91 4.71	2.74 4.33	2.54 3.91	2.228 3.169
11	4.84 9.65	3.98 7.20	3.59 6.22	3.36 5.67	3.20 5.32	3.09 5.07	2.95 4.74	2.79 4.40	2.61 4.02	2.40 3.60	2.201 3.106
12	4.75 9.33	3.88 6.93	3.49 5.95	3.26 5.41	3.11 5.06	3.00 4.82	2.85 4.50	2.69 4.16	2.50 3.78	2.30 3.36	2.179 3.055
13	4.67 9.07	3.80 6.70	3.41 5.74	3.18 5.20	3.02 4.86	2.92 4.62	2.77 4.30	2.60 3.96	2.42 3.59	2.21 3.16	2.160 3.012
14	4.60 8.86	3.74 6.51	3.34 5.56	3.11 5.03	2.96 4.69	2.85 4.46	2.70 4.14	2.53 3.80	2.35 3.43	2.13 3.00	2.145 2.977
15	4.54 8.68	3.68 6.36	3.29 5.42	3.06 4.89	2.90 4.56	2.79 4.32	2.64 4.00	2.48 3.67	2.29 3.29	2.07 2.87	2.131 2.947
16	4.49 8.53	3.63 6.23	3.24 5.29	3.01 4.77	2.85 4.44	2.74 4.20	2.59 3.89	2.42 3.55	2.24 3.18	2.01 2.75	2.120 2.921
17	4.45 8.40	3.59 6.11	3.20 5.18	2.96 4.67	2.81 4.34	2.70 4.10	2.55 3.79	2.38 3.45	2.19 3.08	1.96 2.65	2.110 2.898
18	4.41 8.28	3.55 6.01	3.16 5.09	2.93 4.58	2.77 4.25	2.66 4.01	2.51 3.71	2.34 3.37	2.15 3.01	1.92 2.57	2.101 2.878
19	4.38 8.18	3.52 5.93	3.13 5.01	2.90 4.50	2.74 4.17	2.63 3.94	2.48 3.63	2.31 3.30	2.11 2.92	1.88 2.49	2.093 2.861
20	4.35 8.10	3.49 5.85	3.10 4.94	2.87 4.43	2.71 4.10	2.60 3.87	2.45 3.56	2.28 3.23	2.08 2.86	1.84 2.42	2.086 2.845
21	4.32 8.02	3.47 5.78	3.07 4.87	2.84 4.37	2.68 4.04	2.57 3.81	2.42 3.51	2.25 3.17	2.05 2.80	1.81 2.36	2.080 2.831
22	4.30 7.94	3.44 5.72	3.05 4.82	2.82 4.31	2.66 3.99	2.55 3.75	2.40 3.45	2.23 3.12	2.03 2.75	1.78 2.30	2.074 2.819
23	4.28 7.88	3.42 5.66	3.03 4.76	2.80 4.26	2.64 3.94	2.53 3.71	2.38 3.41	2.20 3.07	2.00 2.70	1.76 2.26	2.069 2.807

APPENDIX III—*contd.*

		Degrees of freedom for greater mean square										Values of <i>t</i>
		1	2	3	4	5	6	8	12	24	$\infty$	
Degrees of freedom for smaller mean square	24	4.26 7.82	3.40 5.61	3.01 4.72	2.78 4.22	2.62 3.90	2.51 3.67	2.36 3.36	2.18 3.03	1.98 2.66	1.73 2.21	2.064 2.797
	25	4.24 7.77	3.38 5.57	2.99 4.68	2.76 4.18	2.60 3.86	2.49 3.63	2.34 3.32	2.16 2.99	1.96 2.62	1.71 2.17	2.060 2.787
	26	4.22 7.72	3.37 5.53	2.98 4.64	2.74 4.14	2.59 3.82	2.47 3.59	2.32 3.29	2.15 2.96	1.95 2.58	1.69 2.13	2.056 2.779
	27	4.21 7.68	3.35 5.49	2.96 4.60	2.73 4.11	2.57 3.78	2.46 3.56	2.30 3.26	2.13 2.93	1.93 2.55	1.67 2.10	2.052 2.771
	28	4.20 7.64	3.34 5.45	2.95 4.57	2.71 4.07	2.56 3.75	2.44 3.53	2.29 3.23	2.12 2.90	1.91 2.52	1.65 2.06	2.048 2.763
	29	4.18 7.60	3.33 5.42	2.93 4.54	2.70 4.04	2.54 3.73	2.43 3.50	2.28 3.20	2.10 2.87	1.90 2.49	1.64 2.03	2.045 2.756
	30	4.17 7.56	3.32 5.39	2.92 4.51	2.69 4.02	2.53 3.70	2.42 3.47	2.27 3.17	2.09 2.84	1.89 2.47	1.62 2.01	2.042 2.750
	35	4.12 7.42	3.26 5.27	2.87 4.40	2.64 3.91	2.48 3.59	2.37 3.37	2.22 3.07	2.04 2.74	1.83 2.37	1.57 1.90	2.030 2.724
	40	4.08 7.31	3.23 5.18	2.84 4.31	2.61 3.83	2.45 3.51	2.34 3.29	2.18 2.99	2.00 2.66	1.79 2.29	1.52 1.82	2.021 2.704
	45	4.06 7.23	3.21 5.11	2.81 4.25	2.58 3.77	2.42 3.45	2.31 3.23	2.15 2.94	1.97 2.61	1.76 2.23	1.48 1.75	2.014 2.690
	50	4.03 7.17	3.18 5.06	2.79 4.20	2.56 3.72	2.40 3.41	2.29 3.19	2.13 2.89	1.95 2.56	1.74 2.18	1.44 1.68	2.008 2.678
	60	4.00 7.08	3.15 4.98	2.76 4.13	2.52 3.65	2.37 3.34	2.25 3.12	2.10 2.82	1.92 2.50	1.70 2.12	1.39 1.60	2.000 2.660
	70	3.98 7.01	3.13 4.92	2.74 4.07	2.50 3.60	2.35 3.29	2.23 3.07	2.07 2.78	1.89 2.45	1.67 2.07	1.35 1.53	1.994 2.648
	80	3.96 6.96	3.11 4.88	2.72 4.04	2.49 3.56	2.33 3.26	2.21 3.04	2.06 2.74	1.88 2.42	1.65 2.03	1.32 1.49	1.990 2.638
	90	3.95 6.92	3.10 4.85	2.71 4.01	2.47 3.53	2.32 3.23	2.20 3.01	2.04 2.72	1.86 2.39	1.64 2.00	1.30 1.45	1.987 2.632
	100	3.94 6.90	3.09 4.82	2.70 3.98	2.46 3.51	2.30 3.21	2.19 2.99	2.03 2.69	1.85 2.37	1.63 1.98	1.28 1.43	1.984 2.626
	125	3.92 6.84	3.07 4.78	2.68 3.94	2.44 3.47	2.29 3.17	2.17 2.95	2.01 2.66	1.83 2.33	1.60 1.94	1.25 1.37	1.979 2.616
	150	3.90 6.81	3.06 4.75	2.66 3.91	2.43 3.45	2.27 3.14	2.16 2.92	2.00 2.63	1.82 2.31	1.59 1.92	1.22 1.33	1.976 2.609
	200	3.89 6.76	3.04 4.71	2.65 3.88	2.42 3.41	2.26 3.11	2.14 2.89	1.98 2.60	1.80 2.28	1.57 1.88	1.19 1.28	1.972 2.601
	300	3.87 6.72	3.03 4.68	2.64 3.85	2.41 3.38	2.25 3.08	2.13 2.86	1.97 2.57	1.79 2.24	1.55 1.85	1.15 1.22	1.968 2.592
	400	3.86 6.70	3.02 4.66	2.63 3.83	2.40 3.37	2.24 3.06	2.12 2.85	1.96 2.56	1.78 2.23	1.54 1.84	1.13 1.19	1.966 2.588
	500	3.86 6.69	3.01 4.65	2.62 3.82	2.39 3.36	2.23 3.05	2.11 2.84	1.96 2.55	1.77 2.22	1.54 1.83	1.11 1.16	1.965 2.586
	1000	3.85 6.66	3.00 4.63	2.61 3.80	2.38 3.34	2.22 3.04	2.10 2.82	1.95 2.53	1.76 2.20	1.53 1.81	1.08 1.11	1.962 2.581
	$\infty$	3.84 6.64	2.99 4.60	2.60 3.78	2.37 3.32	2.21 3.02	2.09 2.80	1.94 2.51	1.75 2.18	1.52 1.79	1.00 1.00	1.960 2.576

Snedecor's *F* is the same as Mahalanobis'  $\lambda$  and is the ratio of Variance<sub>1</sub> to Variance<sub>2</sub>.

Snedecor, G. W. (1934).—Calculation and Interpretation of Analysis of Variance and Covariance. Iowa, pp. 88-91.



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